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THE GLANDS OF THE BILE AND PANCREATIC DUCTS: AUTORADIOGRAPHIC AND HISTOCHEMICAL STUDIES

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INTRODUCTION

'To obtain any idea of the histologic structure of the bile ducts one must consult books which have long since been swept by in the current of medical literature and are almost forgotten.' This sentence is largely as true to-day as it was when written by Burden in 1925. Since that time the majority of the investigations that have been carried out on the extra-hepatic bile ducts have been concerned with the musculature of the ducts, and its possible functional significance is being studied at the present time by Burnett & Shields (1958). In a recent series of papers, Boyden reported his observations on the choledocho-duodenal junction in a number of laboratory animals and in man (Boyden, 1955, 1957*a*, *b*; Eichhorn & Boyden, 1955), but no attention was paid to the mucous membrane, which was always destroyed during maceration of the specimens. Most text-books of histology make only a passing reference to the glands of the bile and pancreatic ducts. There is general agreement that there are mucous glands in the pancreatic duct, and most authors describe goblet cells as well. As far as the bile duct is concerned, Ham (1957) mentions the presence of tubulo-alveolar glands, which Garven (1957) states are not mucus-secreting, while Mann (1932) subscribes to the presence of glands with both a mucous and serous function. Maximow & Bloom (1957) assert that the columnar epithelium of the extra-hepatic biliary passages yields atypical mucus, and Deane (1954) describes the columnar cells as possessing a striated border and apical mucus droplets, apart from the presence of occasional goblet cells. Trautmann & Fiebiger (1952), dealing with domestic animals, state that mucous glands and goblet cells are found in pancreatic ducts, but that in the bile ducts goblets are found only in the pig, ox and horse; many glands are normally present, except in the pig.

The present work has been undertaken for the specific purpose of studying the mucosa of the lower ends of the bile and pancreatic ducts where they open into the lumen of the gut, with special reference to the glands that may be found in the walls of those ducts. Morphological studies on laboratory animals and on human specimens from the recently deceased have been supplemented by a number of histochemical observations, and by autoradiography using $^{35}SO_4$ *in vivo* and/or *in vitro*.

MATERIALS AND METHODS

A total of 48 adult animals of the following species was used: mouse (4), hamster (6), rat (11), guinea-pig (9), cat (7), dog (5) and Rhesus monkey (6). They were sacrificed in the morning having had access to water only overnight. The human material

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consisted of seven adult autopsy specimens, removed from 2 to 24 hr. post-mortem. The post-cibal status of the individuals was uncertain.

In the mouse, rat and hamster, the main pancreatic duct joins the bile duct before the latter opens into the small intestine. In the cat, monkey and man, the bile and main pancreatic ducts normally open on a common papilla, with an adjacent papilla for an accessory pancreatic duct. In the guinea-pig, the main pancreatic duct opens into the duodenum about 7 cm. distal to the bile duct, whose opening is very near the pylorus. In the dog, the main pancreatic duct opens distal to a papilla that receives the bile and accessory pancreatic ducts.

For this investigation, the regions of the gut bearing the duct openings were removed together with an adjacent segment of the appropriate duct or ducts. The fixatives used included neutral formalin, ice-cold 80% alcohol and Gendre's and Rossman's fluids; some specimens were freeze-dried or freeze-substituted, and fresh frozen sections were also used.

Apart from routine staining with haematoxylin and eosin, mucicarmine or iron haematoxylin and picrofuchsin (van Gieson), the following histological and histochemical staining procedures were used on selected serial sections:

(1) The cobalt sulphide or azo dye methods for alkaline phosphatase.

(2) The PAS technique, with and without diastase digestion, for protein-carbohydrate complexes and glycogen.

(3) Alcian blue, Hale's colloidal iron or toluidine blue for acid mucopolysaccharides.

(4) Toluidine blue or methyl green-pyronin, with and without ribonuclease digestion, for cytoplasmic basophilia, indicating ribonucleoprotein (RNA).

(5) The neotetrazolium method for succinic dehydrogenase carried out on fresh frozen tissue under anaerobic conditions, as an index of cellular metabolic activity.

Autoradiographic technique

For studies *in vivo*, animals were injected intraperitoneally with carrier-free $Na_2{}^{35}SO_4$, in doses of 4 μc./g. body weight for the mouse, 2 μc./g. for the hamster, and 1 μc./g. for the rat and guinea-pig. One cat of 2·5 kg. received a dose of 9 mc. The animals were killed 6 hr. after the administration of the isotope. For *in vitro* studies, pieces of duct tissue approximately 2 mm. long and 0·5 mm. thick from the hamster, rat, guinea-pig, cat, dog, monkey and man were incubated in roller tubes for 6 or 12 hr. at 37° C. in 2 ml. Tyrode's solution containing 2 μc. of radio-sulphate, 200 units of penicillin and 200 units of streptomycin (Curran & Kennedy, 1955).

Autoradiographs were prepared using the stripping-film technique (Heatley, Jerrome, Jennings & Florey, 1956), the exposure times being from 3 to 6 weeks.

RESULTS

The cytological findings in the duct epithelium and glands are detailed below under species headings. In general, it was noted that in any given animal, there were negligible morphological differences between the epithelial cells lining the bile and pancreatic ducts, or between the duct cells and those of the ampullae and papillae. However, the transition between papillary and intestinal epithelium was very

abrupt (Pl. 1, fig. 1). Also, there was considerable species variation in the size of the epithelial cells lining the ducts, those of the guinea-pig being by far the largest (Pl. 2, cf. figs. 14, 15).

Unless otherwise stated, material that was PAS-positive also gave positive reactions with Alcian blue and Hale's colloidal iron.

Mouse

During its course through the pancreas the bile duct proved to be a straight-walled tube, lined by columnar epithelium containing rounded basal nuclei. A number of small ducts from the pancreas opened into the bile duct at irregular intervals. On piercing the intestinal wall, numerous sac-like glands opened into the lumen of the duct (Pl. 2, fig. 9), and these were especially frequent near the papilla. Very occasional mitotic figures were noted in the duct epithelium.

Staining with mucicarmine and with the PAS technique revealed the presence of goblet cells scattered throughout the epithelium of the duct. The epithelial cells themselves and those of the glands stained strongly at their luminal borders and very often also in the supranuclear regions. The PAS-positive material was diastase-resistant. No metachromasia was noted, except in mast cells that were occasionally found in the periductal connective tissue. Compared with intestinal and pancreatic cells, the cells of the duct and glands contained only small amounts of RNA. Alkaline phosphatase activity was confined to a small number of stromal blood vessels, but a strongly positive reaction for succinic dehydrogenase was present in all epithelial cells.

Autoradiographs of ducts from animals that had received $^{35}SO_4$ showed that strong radioactivity was present in the goblet cells of the ducts and in most of the glands, but that there was no activity in the other epithelial cells.

Hamster

In the hamster, the distal end of the bile duct in its extra-intestinal course possessed a smooth lining of columnar cells without any glandular downgrowths. After piercing the muscular wall of the gut and while traversing the intestinal mucous membrane, the duct lining became thrown into folds but no diverticula comparable to those noted in the mouse were seen. Staining with mucicarmine and with the PAS method revealed many goblet cells in the duct lining; the remaining columnar cells possessed luminal borders and supranuclear regions that were PAS-positive. Diastase digestion did not alter the extent of PAS staining, hence none of this material could be identified as glycogen. The sites of PAS-positive material also gave a metachromatic reaction with toluidine blue. Some mast cells were noted in the periductal connective tissue. Treatment with ribonuclease removed the cytoplasmic basophilia of the duct cells almost completely, though some coloration persisted in the goblet cells and in the luminal borders. No alkaline phosphatase was found either in the duct epithelium or in its underlying connective tissue framework, but the epithelium gave a strongly positive reaction for succinic dehydrogenase.

Autoradiographs of duct tissue revealed strong activity in the mucin of the goblet

cells, and also in the borders and supranuclear regions of the remainder of the lining cells. Precisely similar activity was found in portions of duct that had been cultured for 6 or 12 hr., the longer period merely emphasizing the activity that could be seen after 6 hr.

Rat

The epithelial lining of the bile duct in the rat consisted of columnar cells, with a number of goblets that were less numerous than in the hamster and were chiefly found near the papilla. As the duct approached the intestine it was characterized by the presence of sac-like diverticula that became more numerous and test-tube-like as the duct pierced the intestinal wall. These downgrowths were lined by typical columnar duct cells interspersed with goblets. The goblets of the duct and in the downgrowths were PAS and mucicarmine-positive, and also showed metachromasia, but in the columnar cells themselves the luminal borders were only very faintly PAS-positive (Pl. 2, fig. 14). No significant staining reactions were noted in the supranuclear regions of these cells; there was no staining with mucicarmine, Alcian blue or Hale's method and no metachromasia. None of the PAS-positive material was removed by diastase digestion, but ribonuclease removed almost all the cytoplasmic basophilia of the columnar cells. Blood vessels in the subepithelial connective tissues contained alkaline phosphatase, but this enzyme was not observed in the epithelium. However, succinic dehydrogenase was present, both in the duct lining itself and in the diverticula.

Radioactivity in the duct and its glands both *in vivo* and *in vitro* was confined to the goblet cells, the ordinary epithelial cells (as in the mouse) showing no evidence of having incorporated the sulphur isotope (Pl. 1, fig. 2).

Guinea-pig

In this species the terminations of both the bile and pancreatic ducts were available for study, but the two ducts were essentially similar in structure, differing only in quantitative respects.

Both ducts were lined by columnar cells interspersed with goblets that were more numerous in the pancreatic than in the bile duct. The lining cells showed PAS-positive, diastase-resistant borders and supranuclear regions. The goblet cells were usually but not invariably metachromatic, and the cytoplasmic basophilia of the columnar cells was almost completely removed by digestion with ribonuclease.

Opening into the lumen of the intra-mural and the adjacent extra-mural portions of both ducts was a large number of tubulo-alveolar glands (Pl. 1, fig. 3). The bile duct was somewhat thicker in section than the pancreatic duct due to the greater depth of these glands in the former. The upper, tubular parts of the glands were lined by columnar cells and goblets that gave the same histochemical reactions as the epithelium of the duct lining (Pl. 1, fig. 6). The lower parts appeared as typical glandular alveoli whose cells were packed with PAS and mucicarmine-positive material that was also metachromatic (Pl. 1, figs. 4 and 6–8). In some specimens the more superficial portions of the alveoli, while still PAS-positive, did not stain with mucicarmine and showed no metachromasia. Alkaline phosphatase was absent from the duct and its glands, but there was strong succinic dehydrogenase activity.

Radioactivity was present in both ducts in the mucin of the goblet cells, but was strongest in the basal parts of the alveoli (Pl. 1, fig. 3), i.e. the parts that were constantly metachromatic and mucicarmine-positive as well as PAS-positive. The remaining more superficial parts of the alveoli did not exhibit radioactivity.

Cat

The most striking cytological feature in this species when compared with the rodents examined was the complete absence of goblet cells from all the ducts (Pl. 2, fig. 10). The bile and pancreatic ducts, opening on their common papilla through the ampulla, were lined as in the rodents by columnar cells with borders and supranuclear regions that were PAS and mucicarmine-positive, but there was no metachromasia. Many mucosal folds were present in the ampullary and papillary regions. A number of sac-like glands opened into the lumina of both ducts (Pl. 2, fig. 10). They contained no goblet cells, but those in the pancreatic duct nearest the papilla contained alkaline phosphatase in their cytoplasm. The enzyme was not seen in the glands of the bile duct itself, but those glands that opened into the ampulla common to both ducts gave a strongly positive phosphatase reaction (Pl. 2, fig. 11). The cells of the glands of both the ducts and the ampulla usually stained with PAS and mucicarmine, and a considerable amount of RNA was present. In one specimen a number of epithelial cells, on the very summit of the papillary opening into the lumen of the gut, contained perinuclear granules of glycogen. This was the only instance throughout the course of the present investigation in which glycogen was detected.

Autoradiographs of portions of cultured ducts revealed an uptake of sulphur by all the epithelial cells lining the ducts, and radioactivity was usually but not invariably present in the glands (Pl. 2, fig. 17). A precisely similar distribution, but of greater intensity, was noted in the duct tissues of the cat that had received a large dose of isotope *in vivo*.

Dog

As in the cat, numerous folds of mucous membrane were found in the lower ends of the three principal ducts and their associated ampullae and papillae (Pl. 1, fig. 5). The columnar epithelial cells in this species were particularly tall and closely packed (Pl. 2, fig. 16), though in the many saccular glands that opened into the lumina of the ducts they were more cubical in form. Mitotic figures were frequent, especially in the epithelium of the papillary regions, and numbers of infiltrating lymphocytes were also noted.

The cells of both the ducts and glands were PAS and mucicarmine-positive, and occasionally but not always exhibited metachromasia. In one dog the glands associated with the bile duct did not stain with Alcian blue or mucicarmine, although they were PAS and Hale-positive. No goblet cells were found in any of the ducts (Pl. 1, fig. 5), but, unlike any other species examined, the cells lining all the ducts, as well as those in the glands, gave a strongly positive reaction for alkaline phosphatase (Pl. 2, fig. 16). Succinic dehydrogenase was also present in duct lining cells and glands.

Culture of the ducts with radio-sulphur showed that the cells lining all the ducts

were radioactive at their luminal borders. Only a few of the glands were overlaid by granules in the autoradiographs, and such radioactivity as was present was in the superficial and not in the deep portions.

Monkey

The general morphological pattern of the ducts closely resembled that of the cat and dog. Sac-like glands were present in the bile, pancreatic and accessory pancreatic ducts. The columnar epithelial lining of the ducts and glands contained goblet cells that were much more abundant in the pancreatic than in the bile ducts. The mucus of the goblet cells, together with the borders and supranuclear regions of many of the columnar cells, stained deeply with PAS, and rather faintly with mucicarmine, metachromasia being often but not constantly present in these regions. Alkaline phosphatase was not present in duct or glandular epithelium, but was found in the stroma surrounding the papilla and in some subepithelial and periglandular blood vessels.

Following culture with radioactive sulphur, autoradiographs revealed activity in the luminal borders of the duct epithelium (Pl. 2, fig. 18), and in the region of the necks of the glands but not at deeper levels.

Man

In all the human specimens, even that removed 2 hr. post-mortem, most of the epithelium lining the lower ends of the main ducts had undergone autolysis and only small fragments were available for study. However, the glands appeared to be well preserved in most instances in both bile and pancreatic ducts.

The available surface epithelium was of the tall columnar variety, and the luminal borders and supranuclear regions stained strongly with PAS and mucicarmine. No metachromasia was encountered, nor were any goblet cells found. Ribonuclease removed most of the cytoplasmic basophilia. The glands, as previously noted by Burden (1925), were arranged in irregular groups and not evenly distributed throughout the mucosa as was the case in all other species. Tubulo-alveolar in nature, their epithelium was more cuboidal than that of the duct lining itself, but gave the same staining reaction, without metachromasia or goblet cells (Pl. 2, fig. 12). No epithelial or glandular pathology was found in the present series, although studies by Bagenstoss (1938) and Birnstingl (1959) on much larger numbers of specimens indicate that minor pathological processes such as epithelial hyperplasia or metaplasia are not uncommon. Alkaline phosphatase was present only in mucosal blood vessels, but both surface epithelium and glands contained succinic dehydrogenase (Pl. 2, fig. 19).

Most but not all of the glands in the bile and in the pancreatic ducts showed radioactivity after culture with $^{35}SO_4$ (Pl. 2, fig. 13). No conclusions about sulphate in the lining cells could be reached owing to the unsatisfactory nature of the particular specimens incubated.

DISCUSSION

These studies reveal morphological differences between species with regard to the lower ends of the bile and pancreatic ducts and their associated glands. The simplest bile duct of this series is that of the hamster—a straight tube, the mucosa being thrown into folds only a little distance from the papillary opening. The most complex is that of the guinea-pig, which like the pancreatic duct in this species possesses a thick mucosa due to the presence of many tubulo-alveolar glands.

The histochemical findings indicate that all the ducts are capable in some way or other of secreting protein-carbohydrate complexes, some of which is mucin that can be shown by autoradiography to be of the sulphated variety, though the sites of origin of these secretions show species differences. In the rat, goblet cells appear to be the sole source of duct secretion, whereas in all other species examined the epithelial cells lining the ducts are secretory. The cat and dog are the only species that do not possess goblets, but our human material does not show goblet cells either. A larger series of fresh specimens is required before a definite answer can be given to this question in man. The hamster alone possesses no glands comparable with any other species, although in the rat there are no alveoli and the 'glands' are simply downgrowths of lining epithelium containing scattered goblet cells.

The autoradiographic evidence indicates that some at least of the mucin secreted by the ducts is of the sulphated variety. The autoradiographs of cultured material from the smaller animals give results that very closely resemble those obtained from the same animals *in vivo*, and it may be that the ducts in question could well be added to the structures listed by Trowell (1959) as suitable for organ culture. With the reservation that the behaviour of cells in culture has not always proved to be a reflexion of their behaviour *in vivo*, it is considered reasonable to assume that the *in vitro* findings in the dog, monkey and man give sufficient indication of whether or not duct cells and glands are capable of secreting sulphated material, although whether the sulphur in culture is being actively metabolized or merely exchanged is a question that need not be debated here.

All the goblet cells of this series show radioactivity following the administration of $^{35}SO_4$, and in this respect they are identical with the goblets of the intestine (Jennings & Florey, 1956) and of the gall-bladder and trachea of some species (Jennings, 1958). Wherever alveolar glands are present, i.e. in all species except the rat and hamster, some or all of their cells show radioactivity. Although, like all other species except the rat, the lining cells of the mouse and guinea-pig ducts are secretory, as evidenced for example by positive staining with PAS, Alcian blue and Hale's colloidal iron, there is no suggestion of radioactivity in these cells, whereas those of the hamster, cat, dog, monkey and man do give positive autoradiographs. In some situations, e.g. in most goblet cells, the site of formation of sulphated mucin can be correlated with metachromasia and positive staining with mucicarmine, but in other situations this is not always so. For example, in some guinea-pig specimens, where only the deeper portions of the alveoli are radioactive, there is in these deeper parts metachromasia and positive mucicarmine staining, while in more superficial parts there is no metachromasia, no mucicarmine staining and no radioactivity. But in the glands of the mouse, cat and dog, sites that are mucicarmine

positive and radioactive are not metachromatic. Many factors, as yet little understood, influence the demonstration of metachromasia (cf. the discrepancies noted by Jennings & Florey, 1956), and the interest that is being taken in this matter is reflected in recent papers dealing with its fundamental aspects (e.g. Walton & Ricketts, 1954; Bergeron & Singer, 1958). Furthermore, Pearse (1960) points out that the former belief that the presence of metachromasia indicated specific chemical groups is now untenable. The precise chemical structure of the various forms of mucopolysaccharide still remains to be elucidated despite recent progress (Wolstenholme & O'Connor, 1958; Young & Maw, 1958), and their function too is largely speculative. The evidence suggests that mucins serve as a protective coating for body surfaces (Florey, 1955). If they exercise this function in bile and pancreatic ducts, they are warding off possible injury to the mucosa caused by ascending duodenal contents, or even by the normal hepatic and pancreatic secretions. While some mucins may aid bacterial growth, others may inhibit the proliferation of micro-organisms, and this is a possible additional form of protection.

Glycogen is found in the duct epithelium of only one animal in the present series—a cat—in the form of infranuclear granules in cells on the very summit of the duodenal papilla. From the absence of this substance elsewhere it may be inferred that it is not a normal constituent of duct cell cytoplasm, at least in histochemically detectable quantities. Its presence in one animal is difficult to explain, but it may indicate that the cells in question have regenerated as a result of trauma, for Johnson & McMinn (1960) have shown in the cat that regenerating epithelium in both the small and large intestines is characterized by a small accumulation of glycogen in the new cells within the first week after injury. However, Kugler & Wilkinson (1959, 1960) have suggested that of the two fractions in which cellular glycogen exists—protein-bound and trichloracetic acid-soluble—only the latter fraction is in fact detected histochemically. Thus considerable quantities of glycogen may still be present in cells and yet remain undetected cytologically. The factors influencing a change from one form to another are not known.

While it is well established that alkaline phosphatase can be found in the smaller blood vessels of many species, as for example those in the subepithelial connective tissue of the rat bile duct, its presence in such striking degree in the ducts and glands of the dog, and in the ampullary and pancreatic glands of the cat, is an unexpected finding, especially in view of its complete absence in all other species examined. The presence of phosphatase in bile and pancreatic ducts in the dog has been noted previously by Gomori (1941), Jacoby (1946) and Jacoby & Martin (1951), and the latter authors also found the enzyme to be absent from guinea-pig ducts. Phosphatase in other epithelial sites, e.g. in the intestine and proximal convoluted tubules of the kidney, has been correlated with absorptive processes, but the absorption at least of water can take place without phosphatase, as in Henle's tubules of the kidney. The reason for the presence of phosphatase in duct linings and glands is so far unknown. It is a notable fact that succinic dehydrogenase has been found in all species examined. The presence of this enzyme indicates that considerable metabolic activity is taking place, and that the ducts are not merely serving as passive conduits. In this respect there is a close resemblance between the ducts under discussion and those of the salivary glands of a number of species (Padykula, 1952;

Hill & Bourne, 1954). Babkin (1950) has reviewed the earlier evidence for the existence of a secretory function on the part of salivary gland ducts, and Grossman & Ivy (1946) suggested that the ducts of the pancreas contributed to the exocrine secretions of that organ. Whether the substance secreted by the salivary duct cells is water alone or some other substance has not been established, but it would appear that a similar activity is occurring in bile and pancreatic ducts. The secretions need not of course be identical in all species. It might be speculated that water is being passed from the blood stream into the ducts in order to dilute bile that has been concentrated in the gall-bladder, but for the fact that in the rat that possesses no gall-bladder there appears to be just as much succinic dehydrogenase activity as in other species. There is a considerable field here for biochemical and physiological investigation. 'Bile' collected from the lower end of the bile duct should not always be assumed to contain merely a mixture of mucin and hepatic secretion, since in the guinea-pig, for example, the extensive glandular arrangements suggest that much more than simply mucin is being secreted by the duct. Studies with the electron microscope would also be of interest, to determine whether microvilli are present at the luminal surface of duct cells, and also to establish whether the basal plasma membranes show the characteristics that have been considered by Pease (1956) to be associated with water transport.

SUMMARY

1. The epithelium and glands at the lower ends of the bile and pancreatic ducts of the mouse, hamster, rat, guinea-pig, cat, dog, Rhesus monkey and man have been studied by various histological and histochemical methods, and also by autoradiography using $^{35}SO_4$ *in vivo* and /or *in vitro*.

2. The ducts of all species were found to be capable of secreting protein-carbohydrate complexes, some of which was mucin of the sulphated variety.

3. The secretions were derived from one or more of three sources—goblet cells, duct epithelium (excluding goblets) and alveolar glands—according to species.

4. Goblet cells were not found in the ducts of the cat or dog, nor in the available human specimens. In all other species goblets were present and secreted sulphated mucin.

5. With the exception of the rat, the cells lining the ducts were secretory in all species, the secretion being sulphated except in the mouse and guinea-pig where it was non-sulphated.

6. The hamster and rat possessed no true alveoli. In all other species examined, alveolar glands were present, secreting both sulphated and non-sulphated material.

7. The ducts of all species showed strong succinic dehydrogenase activity, suggesting that, like those of the salivary glands, the ducts are not merely passive conduits but are actively engaged in transport mechanisms.

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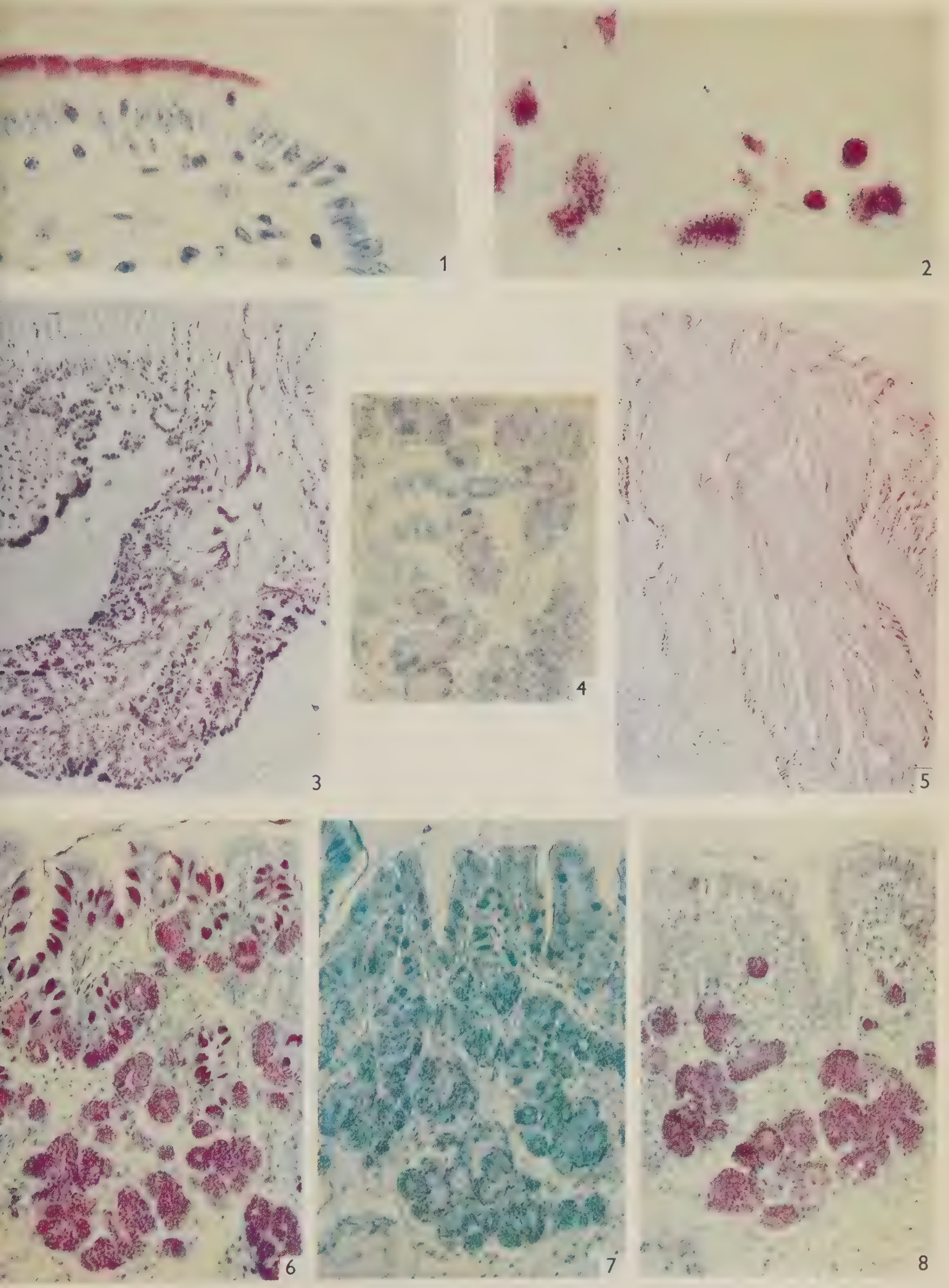

McMINN AND KUGLER—GLANDS OF THE BILE AND PANCREATIC DUCTS

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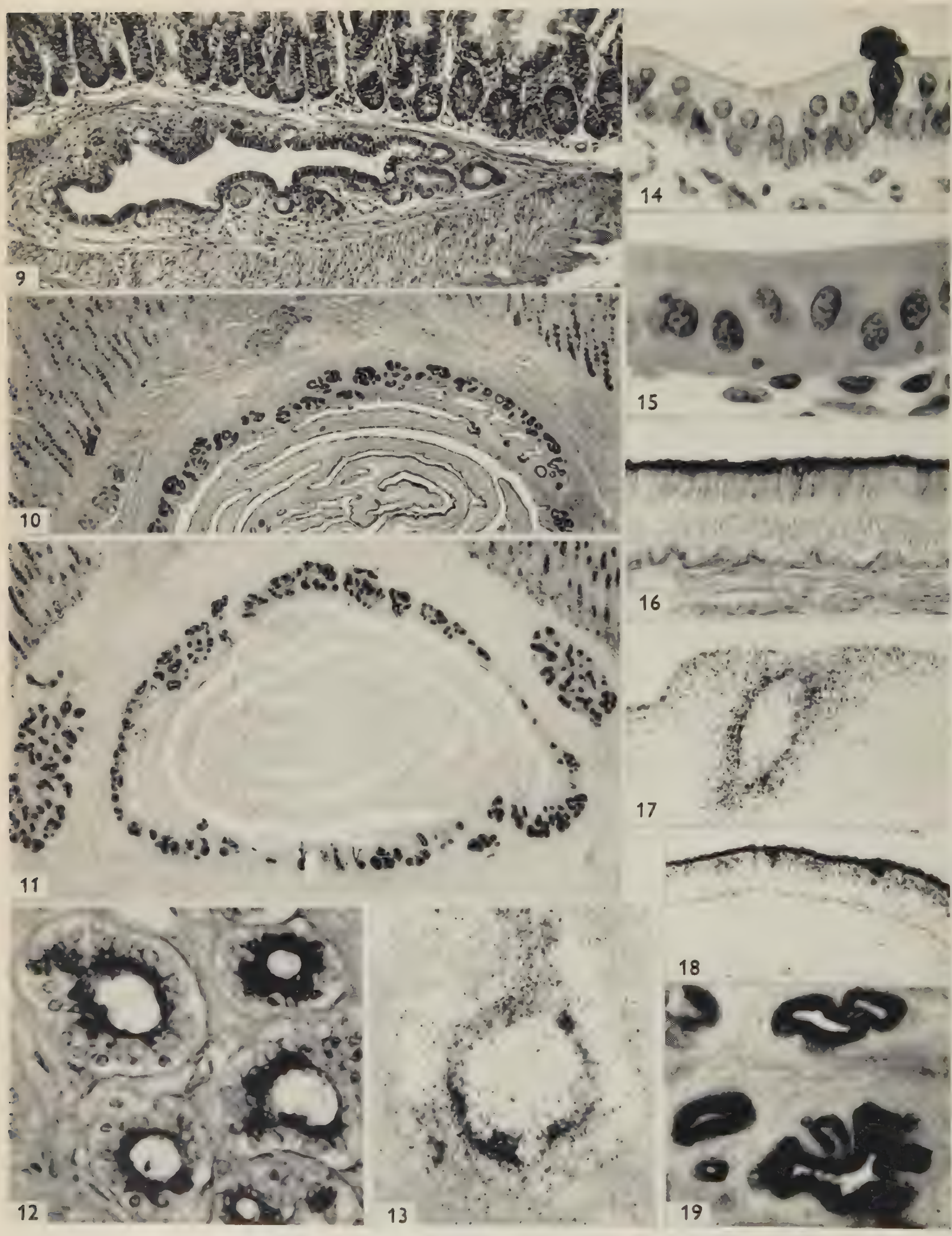

McMINN AND KUGLER—GLANDS OF THE BILE AND PANCREATIC DUCTS

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Cat. Junction between papillary epithelium (on the left) and duodenal epithelium (right). PAS, haematoxylin. ×460.

Fig. 2. Rat. The bile duct surface epithelium (barely visible near the top margin of the figure) shows no radioactivity, whereas the mucin of the goblet cells in the glands gives a strong reaction. Autoradiograph, counterstained PAS. ×480.

Fig. 3. Guinea-pig. Bile duct opening into duodenum. The numerous duct glands show strong radioactivity at their bases, like the pyloric glands of the stomach (upper, left). Autoradiograph, counterstained PAS. ×19.

Fig. 4. Guinea-pig. Glands of the bile duct, showing metachromasia. Toluidine blue. ×58.

Fig. 5. Dog. Accessory pancreatic duct (left) and bile duct opening into the duodenum. Note the deeply staining glands at the periphery of the ducts; compare the duct epithelium with intestinal epithelium (right) and note the absence of goblet cells in the former. PAS, haematoxylin. ×12.

Fig. 6. Guinea-pig. Wall of bile duct, showing lining epithelium with goblet cells, and tubulo-alveolar glands. PAS, haematoxylin. ×58.

Fig. 7. Guinea-pig. As fig. 6. Hale's collodial iron. ×58.

Fig. 8. Guinea-pig. As fig. 6. Mucicarmine. ×58.

Plate 2

Fig. 9. Mouse. Bile duct showing sac-like glands. Haematoxylin and eosin. ×88.

Fig. 10. Cat. Transverse section through half an ampulla showing circumferential glands. Compare ampullary epithelium with that of the intestine (left and upper right) and note the absence of goblet cells in the former. PAS, haematoxylin. ×37.

Fig. 11. Cat. Transverse section through an ampulla. The ampullary glands (and Brunner's glands, left and right) give a strong reaction for alkaline phosphatase, but the ampullary epithelium is negative.

Fig. 12. Man. Glands of the bile duct. PAS, haematoxylin. ×460.

Fig. 13. Man. Gland of bile duct showing maximal radioactivity at the luminal border of the epithelium. Autoradiograph, counterstained PAS. ×510.

Fig. 14. Rat. Epithelium of bile duct. Note the absence of supranuclear and apical staining. PAS, haematoxylin. ×650.

Fig. 15. Guinea-pig. Epithelium of bile duct, at same magnification as fig. 14. Haematoxylin and eosin. ×650.

Fig. 16. Dog. Epithelium of bile duct, showing a strong phosphatase reaction at the luminal border. Gomori technique, incubation time 30 min. ×380.

Fig. 17. Cat. Unstained autoradiograph of bile duct epithelium and gland. ×380.

Fig. 18. Monkey. Bile duct epithelium showing maximal radioactivity in the region of the luminal border. Autoradiograph, counterstained PAS. ×350.

Fig. 19. Man. Glands of the bile duct showing a strong reaction for succinic dehydrogenase. ×30.

THE INFLUENCE OF THE FEMORAL HEAD ON PELVIC GROWTH AND ACETABULAR FORM IN THE RAT

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In order to determine the influence of the femoral head on pelvic growth a series of experiments has been carried out in young rats. The experiments included: (1) unilateral amputation of the hind limb through the hip joint; (2) unilateral amputation just below the femoral neck; (3) unilateral excision of the femoral head; (4) bilateral excision of the femoral head; and (5) unilateral dislocation of the hip joint. It was hoped that the function of the normally situated femoral head would be apparent from analysis of the results of these five different types of experiment. It was also hoped that the results would throw light on the acetabular dysplasia which accompanies congenital dislocation of the hip in man.

There are not many reports of similar studies. Le Damany (1903) demonstrated the importance of normal growth and movements of the femoral head in moulding the acetabulum in the rabbit, and recently Smith, Ireton & Coleman (1958) have described acetabular dysplasia following dislocation of the hip in the growing dog.

MATERIALS AND METHODS

In all eighty-four (61 experimental, 23 litter-mate control) Norwegian rats of the Wistar type were used. The numbers employed in each of the five experiments, the ages at operation, and the survival periods are summarized in Table 1.

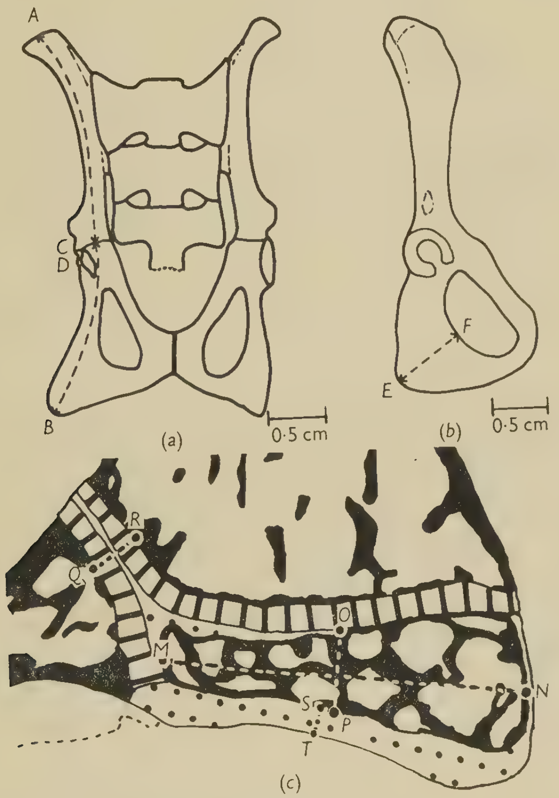
Table 1. *Details of experimental animals used*

Operation	No. of animals used	Age at operation (days)	Survival period (days)
1. Amputation through hip joint	34	4–21	0–365
2. Amputation through femur	6	7–10	53–155
3. Excision of femoral head	11	10–15	24–365
4. Bilateral excision of femoral head	4	10	90–154
5. Dislocation of hip	6	11–15	24–154

The operations were carried out as follows. Amputation through the hip joint was performed through a ventral approach after ligation of the femoral artery. A similar approach was used in excision of the femoral head and in dislocation of the joint. In the last procedure, the joint was dislocated after incision of the capsule, the ligamentum teres severed with a scalpel, and the femoral head displaced to the lateral surface of the ilium. The joint capsule was then sutured to prevent reposition. The animals' gait was observed at intervals after the operation.

The rats were killed with chloroform, after which they were usually radiographed, and then the pelvic region was removed. Approximately half the specimens were

fixed in 10% formol saline or Bouin's fluid, subsequently sectioned serially in paraffin or L.V.N., and stained with Weigert's haematoxylin and van Gieson, Harris's haematoxylin and eosin, or P.A.S.; while the remainder were dissected free of soft parts and studied as gross specimens. In some cases the latter were stained with 0·2% sodium alizarin sulphonate to demonstrate calcified regions more accurately.



Text-fig. 1. Drawings to illustrate the measurements made on the bony specimens and microscopic sections; (*a*) and (*b*) are tracings of the pelvic bones, and (*c*) is an enlargement of the anterior wall of the acetabulum in Text-fig. 3*a*. ×30

Measurements of the curved lengths of the innominate bone (*AB*), ilium (*AC*), and ischium (*BD*), and the maximum width of the ischium (*EF*) were made on the gross specimens (Text-fig. 1*a*, *b*). Measurements made on radiographs were found to be unreliable when compared with those made on gross dissected specimens, especially in older experimental animals with marked asymmetry of the pelvis.

Measurements were also made on most of the histological sections. Sections through the middle of the acetabular cavities were found by examination of the serial sections, and tracings (×70) of these sections in most of the animals in each group were prepared and photographed. From these photographs the diameters and depths of the acetabula were calculated (see Text-fig. 3*a*, *b*, *GH*, *IJ*). Measurements were also made on the stained sections (using a calibrated eyepiece) of the

transverse dimensions of the os acetabuli running through the centre of the bone as shown in Text-fig. 1*c*, *MN*, *OP*, the thickness of the growth cartilages between the components of the innominate bone in the floor of the acetabulum (*QR*), the thickness of the articular cartilage at the middle of the acetabular face of the os acetabuli (*ST*), and the thickness of the bone in the medial wall of the acetabular socket (Text-fig. 3*a*, *b*, *KL*).

RESULTS

(1) *Amputation through the hip joint*

After this operation the animals hopped along on three legs, and a postural scoliosis of the lumbar and sacral parts of the vertebral column concave to the amputated side was obvious 10 days after operation, and this became more marked.

Macroscopic examination of the pelvis showed that the acetabulum as a whole became narrower, shallower, and smaller on the operated side (Text-fig. 2). The brim of the acetabulum on this side gradually lost its sharp edge and regular circular contour, and became blunt and irregularly ovoid, with the long axis running dorso-ventrally. The alizarin-stained areas, representing the calcified cartilage precursors

Text-fig. 2. Drawings of the wax model reconstructions of the acetabula of a rat which had its right hind limb amputated through the hip joint 69 days previously when 21 days old. (*a*) Unoperated side; (*b*) operated side. ×7.

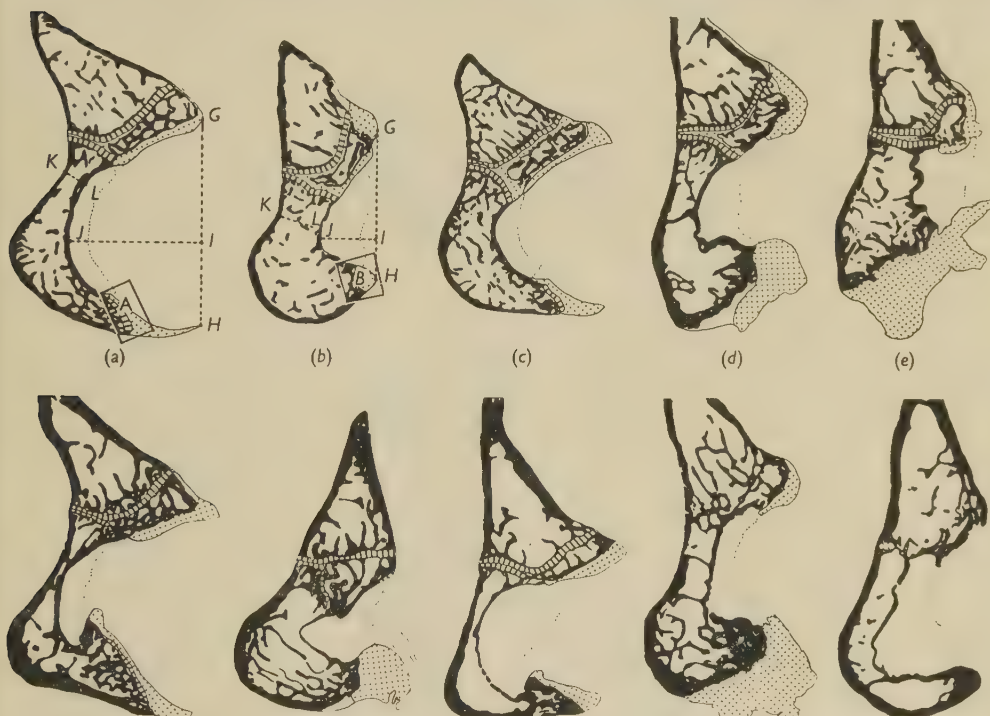
of the os acetabuli and the acetabular epiphysis, appeared at the normal times (24 and 35 days respectively) (Pl. 1, figs. 3, 4). These calcified areas were essentially similar in size, though more irregular in outline, on the amputated side at this stage, indicating that amputation had interfered little with the early stages of maturation of the acetabular cartilage. The corresponding centres of ossification also appeared at the normal times (28 days, and around 70 days, respectively), but were subsequently always smaller on the operated side (Pl. 2, figs. 12, 13; Text-fig. 3*b*, *g*).

Histological examination showed that immediately following operation the torn capsule and the rim of the articular cartilage had become folded into the empty acetabular cavity. Later, the remnants of the subsynovial, synovial and capsular tissues hypertrophied, so that 39 days after amputation the distorted acetabular cavity was filled with fat and sealed off laterally with a thin layer of fibrous tissue.

Reduction in the size and depth of the acetabular socket was very evident in sections taken from the amputated side as was the increased thickness of the bony

floor (medial wall) of the socket where it is formed by non-articular ischium (Text-fig. 3*b*, *g*).

The articular cartilage at the acetabular rim became irregularly thickened and transformed into a disorganized type of fibro-cartilage. Deeper in the cup, e.g. over the os acetabuli, the cartilage was evenly thinner; thus 100 days after operation the cartilage here was one-fifth the thickness of that on the unoperated side, which remained the same thickness as that of a control litter-mate. On the unoperated



Text-fig. 3. Tracings of transverse sections through the centre of the acetabula of rats. Those in the top line are from animals 63 days old, and those in the bottom line are from animals 107 days old. (*a*) and (*f*) are control acetabula; (*b*) and (*g*) show the acetabula following amputation through the hip joint; (*c*) and (*h*) after amputation through the femur; (*d*) and (*i*) following excision of the femoral head; and (*e*) and (*j*) after dislocation of the hip. All these operations had been carried out when the animals were between 7 and 15 days old. Bone is in black, articular and epiphyseal cartilage stippled, and growth cartilage short parallel lines. The lateral limit of the acetabular pad of fat is indicated by an interrupted line. The rectangular area marked *A* is illustrated in Pl. 2, fig. 14, and the area marked *B* in Pl. 2, fig. 15. ×7.

side wide tracts of basophilic P.A.S.-positive matrix separated the isogenous cell groups of the articular cartilage. On the operated side, however, the matrix was much less abundant and gradually lost its basophilia and reaction with P.A.S., and became eosinophilic, while bordering the free surface a zone of flattened closely packed cells developed (Pl. 1, figs. 5, 6). These changes were not conspicuous for the first 4–7 weeks, but became progressively more marked after that.

The iliac, ischial, and pubic growth cartilage plates within the tri-radiate cartilage complex in the medial wall of the acetabular socket (Harrison, 1958) were unaffected either in thickness or in histological structure following amputation; but the ischial growth plate in the lateral 'cup' part of the acetabular complex gradually became disorganized and had virtually disappeared 53 days after operation (Pl. 2, cf. figs. 14, 15).

The trabeculae in the interior of the innominate bone on the operated side gradually diminished in number and thickness. The cortex also became progressively thinner, especially on the lateral side where it formed the acetabular floor (Text-fig. 4*a*, *b*).

Text-fig. 4. (*a*) and (*b*) are tracings of coronal sections through the centre of the acetabula of a rat which had one hind limb amputated through the hip joint, (*a*) operated side, (*b*) unoperated side; (*c*) and (*d*) are tracings of transverse sections through the centres of the acetabula of a rat which had one hind limb amputated through the femur, (*c*) operated side, (*d*) unoperated side. Both animals were operated on when 7 days old and killed when 162 days old. *IS* = ischial shelf. ×7.

No differences of size or histological structure were found between the unoperated acetabula in experimental animals and their normal litter-mate controls, despite the increased strain which must have fallen on the former following unilateral amputation.

A difference in the lengths of the two innominate bones developed, which increased with the length of time after operation. This difference amounted to 2 mm.

102 days after operation, and was wholly accounted for by poor development of the ischial tuberosity on the amputated side (Pl. 1, fig. 7).

The pelvis as a whole became bent to the amputated side, the innominate bone on that side becoming markedly concave laterally. These changes also developed gradually. Thus 16 days after amputation (at the tenth day after birth) there was little change in the appearance of the pelvis, but after 51 days definite asymmetry had developed, and the innominate bone on the amputated side had rotated medially round a longitudinal axis, so that the acetabulum faced ventro-laterally instead of directly laterally as it normally does (Pl. 2, fig. 17). After 1 year the pelvis was grossly asymmetrical, and the innominate bone on the operated side was noticeably thinner than normal (Pl. 1, cf. figs. 1, 2).

This operation also resulted in gradually increasing structural scoliosis, concave to the amputated side, of the lumbar and sacral regions of the vertebral column. This was not very marked in the cleaned specimens up to 150 days after operation, but later became more pronounced, and was conspicuous 1 year after operation (Pl. 1, fig. 1).

(2) *Amputation through the femur*

Following amputation of the hind limb through the femur just below the femoral neck, the animals' gait and vertebral deformity were similar to those present following amputation through the hip joint.

The growth of the femoral head and the acetabular cup were equally and correspondingly retarded, and their mutual articulating surfaces remained congruent. Measurement showed that the acetabular diameter was more affected than the acetabular depth when compared with the opposite side and with normal controls. The acetabular rim remained sharp and circular in outline unlike the rim after amputation through the joint. Although the os acetabuli remained normal in shape on the operated side there was a one-third reduction in its cross-sectional area ($MN \times OP$, Text-fig. 1*c*) 100 days after operation as compared with a two-thirds reduction a comparable time after amputation through the joint. The bony acetabular floor became thinner than normal, but this change was again less than after amputation through the joint (Text-figs. 3*c*, *h*, 4*c*).

On histological examination the articular cartilage appeared completely normal, and in particular there was no alteration in the P.A.S. staining reaction. However, measurement showed that some thinning had taken place, e.g. 100 days after operation it was four-fifths of the normal thickness (compared with only one-fifth normal following amputation through the joint). As before the only abnormality in the growth cartilages in the acetabular region was a premature disappearance of signs of activity in the ischial shelf. Little change in the thickness of the cortical bone of the acetabular floor was apparent, but a definite decrease in the number of cancellous trabeculae was noted (Text-fig. 3*h*).

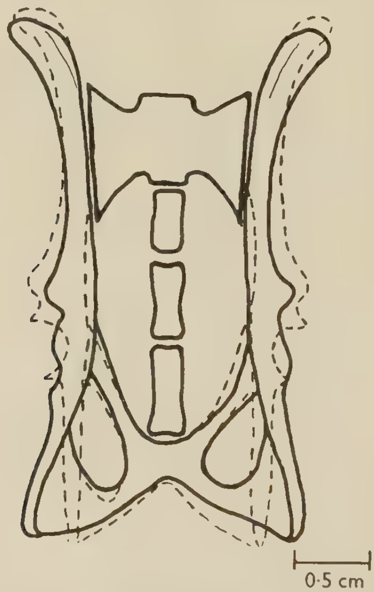
After 150 days a difference in the lengths of the two innominate bones was found. This was of the same order as that found following amputation through the joint, and again it could be wholly accounted for by underdevelopment of the ischial tuberosity on the amputated side. Pelvic asymmetry developed as after amputation through the hip but was less marked (Pl. 2, fig. 16*a*–*c*).

The scoliosis which developed after this operation, however, was similar in degree to that following amputation through the hip joint, up to 150 days after operation, the maximum period of observation.

(3) *Unilateral excision of the femoral head*

Ten days after subcapital excision of the femoral head it was difficult to detect any abnormality of gait. The hind limb was used freely and there was no scoliosis. However, under anaesthesia slight limitation of abduction was present in older animals.

The acetabulum became much shallower than normal but reduction in diameter though present was much less marked than after amputation through the joint (Text-fig. 3*d*, *i*). The acetabular margin became blunt, but the os acetabuli, in keeping with the more nearly normal diameter of the socket, was only slightly reduced in cross-sectional area. The acetabular cavity became filled with fat and



Text-fig. 5. A tracing of the pelvic radiograph of a 154-day old female rat which had both femoral heads removed when 10 days old. Superimposed in interrupted lines is a tracing of the pelvic radiograph of a litter-mate of the same sex.

fibrous tissue. The bone forming the floor of the socket increased in thickness, and the degree of change here was comparable to that following amputation through the joint. The articular cartilage at the rim of the acetabular cavity became thickened and fibrosed, and finally became transformed into fibrous connective tissue which was continuous with the considerable mass of fibrous tissue between the socket and the upper end of the femur. The articular cartilage over the deeper parts of the acetabular cavity underwent changes similar in kind and degree to those described following amputation through the joint. Again the growth cartilage in

the ischial shelf regressed. There was, however, no conspicuous thinning of the innominate bone on the operated side, and no appreciable change in trabecular density.

No difference in the curved lengths of the innominate bones on the two sides was found, but the marked curvature of the operated side gave an apparent shortening when heights were measured by taking the shortest distance between the iliac crest and the ischial tuberosity (Pl. 1, figs. 10, 11).

Asymmetry of the pelvis (i.e. lateral curvature and medial rotation of the innominate bone on the operated side) developed after this operation also, and was conspicuous after 154 days. Scoliosis was not seen even at a year.

(4) *Bilateral removal of the femoral head*

The acetabular changes on the two sides following this operation were similar as would be expected, to those described after unilateral excision of the head.

Both innominate bones became laterally concave and medially rotated around a longitudinal axis, so that the pubic symphysis was flatter than normal. These changes are well seen when the radiographs of normal and experimental pelves are superimposed (Text-fig. 5).

As expected, there was no difference in innominate bone length on the two sides, and no scoliosis developed.

(5) *Dislocation of the hip joint*

Ten days after this operation the animals used the dislocated limb freely and bore weight on it although a slight limp was present. No scoliosis was present.

The acetabular changes closely resembled those seen in specimens where amputation through the joint, or excision of the femoral head, had been carried out (Text-fig. 3*e*, *j*). This applied to the reduction in the size of the socket, blunting of the acetabular margin, reduction in size of the os acetabuli, and thickening of the acetabular floor. As after excision of the femoral head a large amount of fibrous tissue developed around and lateral to the socket. The articular cartilage at the rim of the acetabulum became considerably thickened and fibrosed, while over the os acetabuli it rapidly thinned and was replaced by fibrous connective tissue. The growth cartilage of the ischial shelf was completely disorganized 53 days after operation. The only change in the thickness of the cortex and in the trabeculation of the innominate bone was in the acetabular floor, where the cortex was thinner and the trabeculae less numerous.

There was no difference in the curved lengths of the innominate bones on the two sides. The pelvis became asymmetrical, the innominate bone on the operated side being laterally curved and medially rotated as in the previous experiments. No scoliosis of the vertebral column had developed by 154 days after dislocation (Pl. 1, figs. 8, 9).

CONCLUSIONS AND DISCUSSION

These experiments have shown that marked acetabular dysplasia follows amputation through the hip joint, excision of the femoral head, or dislocation of the hip in the rat (Table 2). Dysplasia is much less in evidence, however, following amputa-

tion through the femur. It would therefore appear that acetabular dysplasia is a consequence of absence of the femoral head from its socket rather than absence of the hind limb as a whole. Pelvic asymmetry parallels acetabular dysplasia, and like the latter appears to be a consequence of the loss of the femoral head from its socket rather than of loss of the limb. This asymmetry took the form of increased lateral curvature and medial rotation of the innominate bone on the operated side. Similar changes were observed on both sides after removal of both femoral heads, indicating that it was removal of the head and not asymmetrical gait which produced the changes in the shape and orientation of the innominate bone. Removal of the leg was responsible for the scoliosis observed in the living animal during locomotion and was due to the asymmetrical gait. This did not occur after excision of the femoral head or dislocation of the hip joint.

Table 2. *Summary of main changes observed after the various experimental procedures*

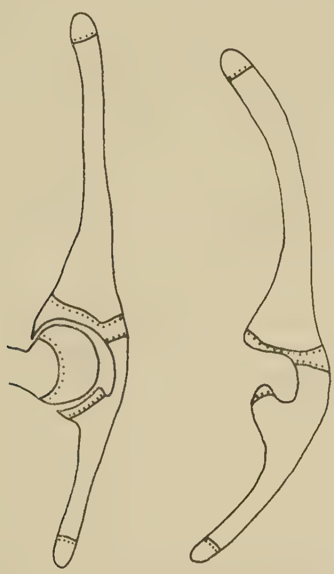
	Amputation through hip joint	Amputation through femur	Excision of femoral head	Dislocation of hip joint
1. Reduction in acetabular diameter	+ + + +	+ +	+ +	+ + +
2. Reduction in acetabular depth	+ + + +	+	+ + +	+ + +
3. Blunting of the acetabular margin	+	0	+	+
4. Reduction in size of the os acetabuli	+ + + + +	+ +	+	+ + +
5. Thickening of the acetabular floor	+ + + + + + + + + +	+ + + + + +	+ + + + + + + + + +	+ + + + + + +
6. Thinning of the central acetabular cartilage	+ + + +	+	+ + + +	+ + + +
7. Reduction in innominate bone length	+	+	0	0
8. Increased curvature of innominate bone	+ +	+	+ +	+ +
9. Presence or absence of scoliosis	+	+	0	0

The number of pluses in 1, 2, 4, 5 and 6 is a measure of the change (one plus = approx. 10 % change) following each operative procedure based on the average measurements on three experimental animals in each group, at survival periods ranging between 50 and 150 days, as compared with three control litter-mates of the same sex.

Disturbances of acetabular development included failure of the socket to develop in area and depth, blunting and irregularity of the acetabular margin, and atrophy and degeneration of the articular cartilage. This latter included loss of matrix basophilia, increase in fibre content, and disorderly arrangement of the cells. In other words the articular cartilage degenerated to a form of fibrous connective tissue. Similar changes at the lower end of the femur have been reported by Bennet, Bauer & Maddock (1932) following amputation through the knee joint with suturing of the synovial membrane over the exposed femoral condyles.

It must be emphasized that these changes were confined to the articular cartilage

of the acetabular cup; the growth cartilages in the tri-radiate medial stem of the cup remained histologically normal and were evidently capable of supporting normal growth, so far as total length of the innominate bone was concerned. (Under-development of the ischial tuberosity following amputation through the hip or femur was no doubt a consequence of the absence of functioning hamstrings.) It is possible, however, that the direction of growth of these cartilages was altered somewhat as a consequence of the collapse of the acetabular cup, thus accounting simply and satisfactorily for the lateral curvature of the innominate bone which developed (Text-fig. 6). Furthermore, as this bone is firmly anchored at the symphysis pubis, collapse of the socket in the dorso-ventral plane would also cause the acetabulum to face ventro-laterally instead of directly laterally. The growth cartilage of the ischial shelf in the caudal wall of the acetabular cup behaved quite differently; it became



Text-fig. 6. Tracings of coronal sections through the innominate bones of a 40-day-old rat which had one hind limb amputated through the hip joint when 4 days old to indicate how collapse of the acetabular cup may alter the direction of growth of the cartilages in its upper and medial walls. The unoperated side has been reversed for comparison. Semi-diagrammatic. $\times 4$.

completely disorganized and eventually ceased to be a growth site. This, however, produced only a local defect in the outline of the bone; over-all growth in length was not involved because this is produced by the growth cartilage in the tri-radiate stem.

The centre for the os acetabuli appeared at the normal time but developed into a smaller, squatter element. This is in keeping with the general finding that the times of appearance of centres of ossification are more rigidly determined genetically than are the size and shape of the resulting bone. It must be mentioned, however, that the experiments were carried out a relatively short time before ossification was due to commence, and interference with the acetabulum at an earlier stage might have produced different results.

The greater over-all width of the bony medial (non-articular) wall of the acetabulum following each type of operation, although here the bone has a thinner cortex and fewer cancellous trabeculae than normal, can be readily explained as a reaction to alterations in the pattern of mechanical stress resulting from absence of the pressure of the femoral head. Le Damany (1903) found similar changes in the bony acetabular floor when for one reason or another pressure between the femur and acetabulum was diminished.

General thinning of the cortex of the innominate bone and diminution in the number of its trabeculae was evident when the limb was removed, especially in older specimens. These findings can be explained by the fact that all the weight from the hind parts was transmitted through the innominate bone on the sound side in the animals with a three-legged gait. The postural scoliosis which developed soon after operation may be explained by muscular imbalance. The fact that structural scoliosis was not present until 4 months after operation is in agreement with the findings of Schwartzmann & Miles (1945), who found that structural scoliosis took a similar time to develop in rats following unilateral removal of the back muscles.

The much greater thickness of the fibrous tissue lateral to the acetabulum in specimens where the femoral head alone had been removed, or where the joint had been dislocated, as compared with specimens in which amputation had been carried out through the joint, may be explained as a consequence of trauma inflicted by the displaced and abnormally mobile upper end of the femur on the capsular tissues.

Recently, Smith *et al.* (1958) have described the macroscopic changes following dislocation of the hip joint in growing dogs. Their radiographs do not show the same degree of pelvic asymmetry as that found in the present experiments, although gross acetabular malformation was present on the operated side. Likewise in a series of experiments by the present author, not previously reported, in which amputation through the joint or excision of the femoral head was carried out on 5-day-old guinea-pigs, pelvic asymmetry did not develop although acetabular malformation was marked. A likely explanation for these differences from the rat is that in the dog and guinea-pig the medial wall of the acetabulum is strong enough to prevent alteration in the alignment of the growth cartilages between ilium, ischium and pubis after loss of the femoral head. In the rat, on the other hand, the acetabular wall is thin and contains a considerable amount of cartilage at the time of operation, so that collapse of the acetabular cup is much more likely to interfere with the alignment of the growth cartilages.

It remains to discuss the acetabular dysplasia observed here in relation to the dysplasia which has been described in congenital dislocation of the hip in man. As the changes observed in unreduced congenital dislocation of the hip (Fairbank, 1930) are very similar to those which follow the removal of the head from its socket in young experimental animals, it is more likely that the dysplasia found in congenital dislocation of the hip is the consequence, rather than the cause, of the dislocation. This is also the opinion of Massie (1956). However, there is not general agreement among pathologists and clinicians, some regarding acetabular dysplasia as the primary cause of congenital dislocation (Hass, 1951), the dysplasia being regarded as a genetic defect (Hart, 1949).

It would appear then that normal postnatal development of the acetabulum depends upon the presence of a normally growing and functioning femoral head. How far this interdependence extends back into foetal life is a matter of considerable theoretical importance. At the shoulder Braus (1909) has shown in amphibia that anlage of the humerus and scapula develop heads and sockets in isolation from each other. Murray (1936) and Badgley (1949), however, conclude from a review of the evidence that only the early form of the joint surface is genetically determined, later growth changes and maintenance of congruence between joint surfaces being dictated by functional stresses.

SUMMARY

The role of the femoral head in pelvic growth in the rat has been investigated by studying the effects of: (1) unilateral amputation of the hind limb through the hip joint, (2) unilateral amputation through the femur, (3) unilateral and (4) bilateral excision of the femoral head, and (5) dislocation of the hip. The effects of these operative procedures have been studied macroscopically, microscopically, and radiographically, and were compared.

Absence of the femoral head from the socket resulted in marked acetabular dysplasia and pelvic asymmetry, while these changes were much less evident when the head was left in the acetabulum. Structural scoliosis developed late after removal of the leg, but did not occur after excision of the femoral head or dislocation of the hip.

It was concluded that normal pelvic growth, especially acetabular development and growth, depends upon a normally placed and growing femoral head, and that the results of these experiments lend support to the view that the acetabular dysplasia found in congenital dislocation of the hip in man is the consequence, rather than the cause, of the dislocation.

I wish to thank Prof. J. J. Pritchard for his helpful discussion of this work and for reading the manuscript. I am indebted to Mr W. T. Haddock for technical assistance, and Mr G. R. Bryan for taking the photomicrographs. My thanks are also due to the Northern Ireland Hospitals Authority for a grant to purchase materials used in this investigation.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. A ventral view of the pelvis of a 367-day-old male rat in which the right hind limb was amputated through the hip joint when the animal was 7 days old. ×1·3.

Fig. 2. A ventral view of the pelvis of a control litter-mate of the same sex as that illustrated in fig. 1. ×1·3.

Fig. 3. A lateral view of the alizarin-stained acetabulum of a 27-day-old rat which had its right hind limb amputated through the hip joint on this side when 11 days old. ×6·5.

Fig. 4. A lateral view of the acetabulum from the unoperated side of the same animal as that shown in fig. 3. ×6·5.

Fig. 5. The area contained in the rectangle in Pl. 2, fig. 12. ×80.

Fig. 6. The area contained in the rectangle in Pl. 2, fig. 13. ×80.

Fig. 7. The innominate bones of a 146-day-old rat which had its left hind limb amputated through the hip joint when 11 days old. ×1·7.

Fig. 8. A radiograph of the pelvis of a 169-day-old rat in which the right hip joint was dislocated when the animal was 15 days old. ×1·0.

Fig. 9. A radiograph of the pelvis of a control litter-mate of the same sex as that shown in fig. 8. ×1·0.

Fig. 10. A ventral view of the alizarin-stained pelvis of a 164-day-old rat which had its right femoral head excised when 10 days old. ×1·3.

Fig. 11. A ventral view of the alizarin stained pelvis of a control litter-mate of the same sex as that shown in fig. 10. ×1·3.

Plate 2

Fig. 12. A transverse section through the centre of the acetabulum of a 90-day-old rat which had its hind limb amputated through the hip joint on this side when 21 days old. Weigert's haematoxylin, van Gieson, and methylene blue. ×17·6.

Fig. 13. A transverse section through the centre of the acetabulum on the unoperated side of the same animal as shown in fig. 12. Weigert's haematoxylin, van Gieson, and methylene blue. ×17·6.

Fig. 14. A photomicrograph of the area contained in the rectangle marked *A* in Text-fig. 3*a*. Weigert's haematoxylin and van Gieson. ×97·4.

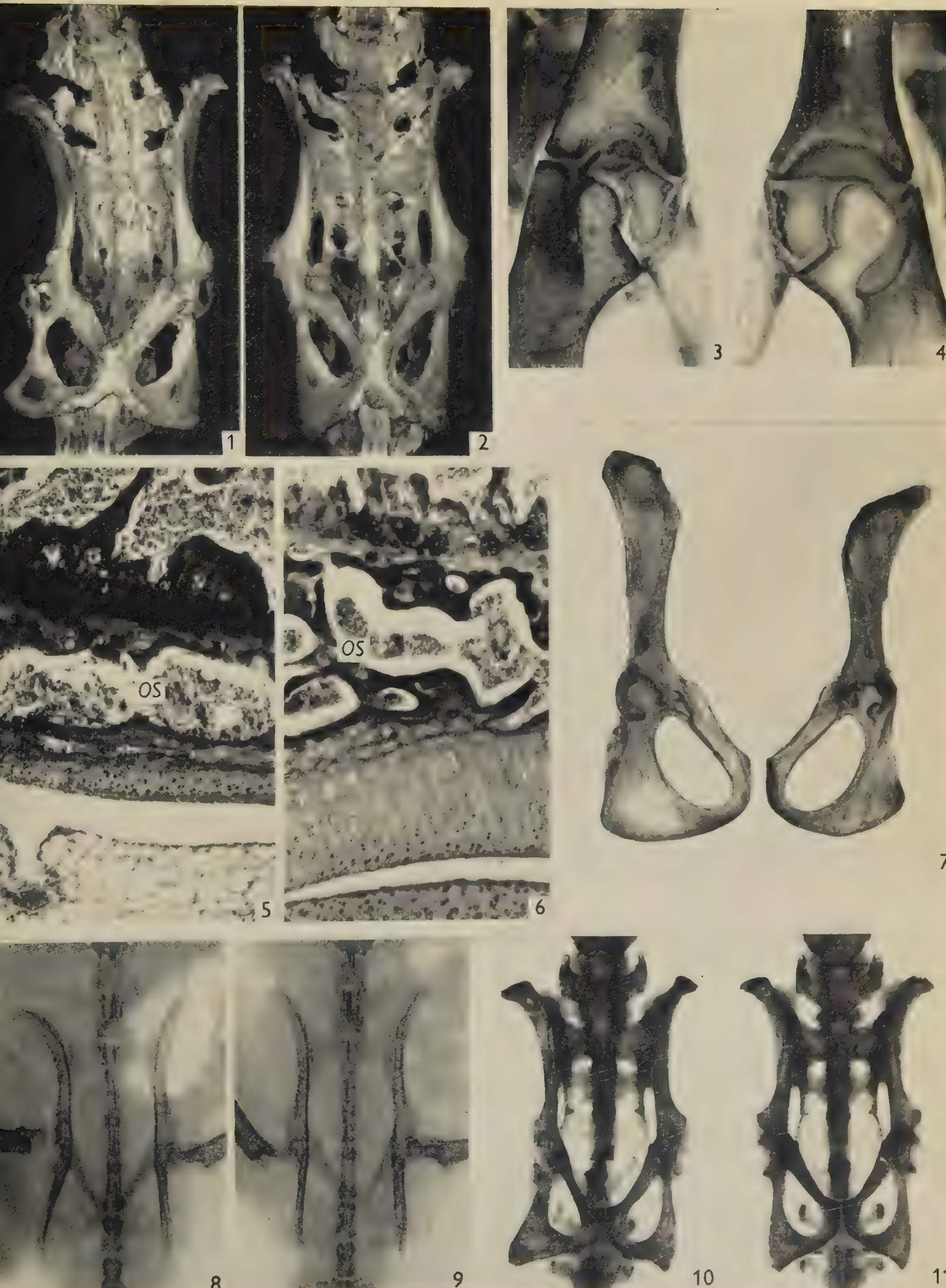
Fig. 15. A photomicrograph of the area contained in the rectangle marked *B* in Text-fig. 3*b*. Weigert's haematoxylin and van Gieson. ×97·4.

Fig. 16. Ventral views of the pelves of three 109-day-old litter-mate rats. (*a*) Control specimen; (*b*) Right hind limb amputated through the femur when 7 days old; (*c*) Right hind limb amputated through the hip joint at the same age. ×1·2.

Fig. 17. A ventral view of the alizarin stained pelvis of a 62-day-old rat which had its right hind limb amputated through the hip joint when 11 days old. ×1·5.

Key to lettering

OS, os acetabuli; *AE*, commencing ossification for acetabular epiphysis.



HARRISON—INFLUENCE OF THE FEMORAL HEAD ON PELVIC GROWTH

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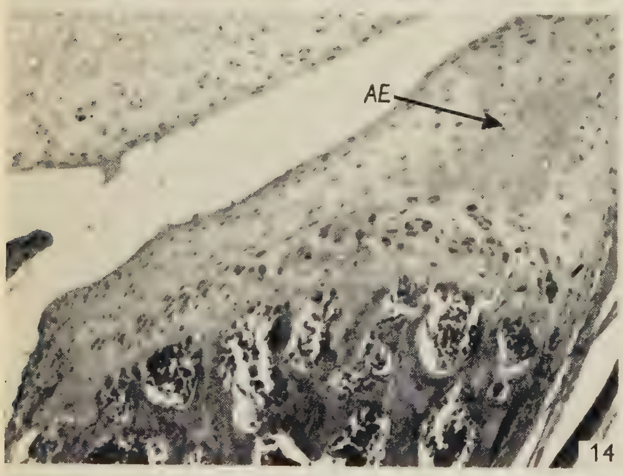

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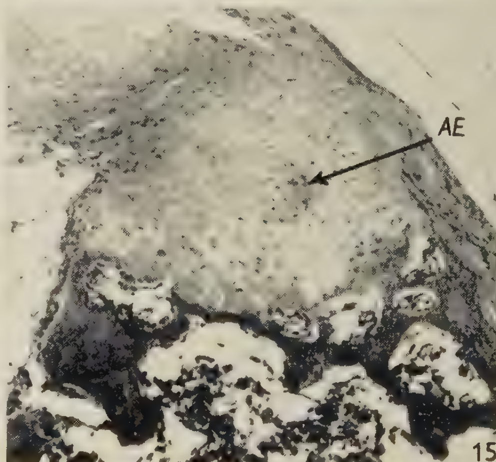

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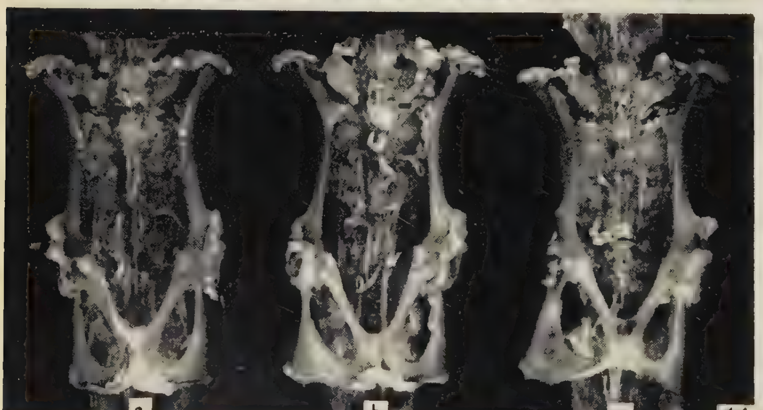

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HARRISON—INFLUENCE OF THE FEMORAL HEAD ON PELVIC GROWTH

MUSCLE SPINDLES IN HUMAN LARYNGEAL MUSCLES

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INTRODUCTION

This report of an investigation relating to nerve endings in human laryngeal muscles forms part of a wide comparative study of the nerve supply of the larynx planned by Prof. R. E. M. Bowden and supported by a grant from the Medical Research Council. This study is especially directed to determine the presence or absence of neuromuscular spindles in these muscles in man, but does not include examination of the size-frequency distribution of fibre sizes in the nerves to individual muscles, nor the disposition of the spindles within the muscles.

MATERIAL AND METHODS

The material consists of muscles taken from two larynges which were kindly supplied by the Pathology Department of the Royal Free Hospital. From one specimen two pairs of whole muscles have been cut in serial section, mounted and stained. These are the cricothyroid and the cricoarytenoideus posterior muscles. One of each pair was cut longitudinally, the other transversely to the fibres. Tags of arytenoideus, thyroarytenoideus and of cricoarytenoideus lateralis were left attached to the left cricoarytenoideus posterior which was cut transversely, and these portions of muscles have been examined. From the other larynx only the left cricothyroid cut transversely in serial section has been examined.

All the muscles were cut at 15μ, and every section was mounted. Alternate slides of one of the blocks (the left cricothyroid) were stained by Romanes (1950) silver method, and haematoxylin and Biebrich scarlet. All the other sections were stained by Romanes silver method. The collection comprises 980 slides, presenting roughly 5000 sections.

It is difficult to obtain really fresh material even from the operating theatre, and the material used in the present work came from the post-mortem room, so inevitably the more exposed parts of the muscles suffered deterioration. Thus it is difficult in studying the sections of the above series to determine accurately the disposition of spindles within the muscles, but possibly, after further work, an over-all picture may emerge.

OBSERVATIONS

A preliminary review of the whole of the material makes it clear that neuromuscular spindles are present in all the laryngeal muscles of man in considerable numbers, and also that these spindles exhibit the basic pattern described by Sherrington in 1894.

Spindles are easily identified in transverse sections of the muscle fibres (Pl. 1, figs. 1–3), but it is disappointing that though many portions of spindles are to be

seen cut in longitudinal sections of the muscle fibres, only one complete spindle cut through its whole length has as yet been demonstrated (Pl. 1, fig. 4).

The compound spindle cut in transverse section (Pl. 1, fig. 1) and traced throughout its course measures 1·41 mm. in length, and passes through 94 sections.

Spindles were also found in the tags of muscles attached to the main block of the left cricoarytenoideus posterior, namely in cricoarytenoideus lateralis (Pl. 1, fig. 5), thyroarytenoideus (Pl. 1, fig. 6) and arytenoideus (Pl. 1, fig. 7).

Other sensory nerve endings are seen; for example, a claw ending (Pl. 1, fig. 8) and a spiral nerve ending (Pl. 1, fig. 9).

Motor end plates are numerous and are demonstrated more easily in sections of muscle fibres cut in their length rather than in transverse sections of the muscle fibres (Pl. 1, fig. 10). Several end-plates were observed to be served by two fibres, one coarse and one fine (Pl. 1, fig. 11). Pl. 1, fig. 12 shows a coarse nerve serving a motor end-plate and a fine fibre supplying a capillary, and Pl. 1, fig. 13, demonstrates a nerve fibre supplying two end-plates.

DISCUSSION

The question relating to the presence or absence of neuromuscular spindles in certain groups of non-skeletal striped muscles in various animals has excited considerable interest since the last decade of the nineteenth century.

These groups of muscles include those of the face, the tongue, the extrinsic muscles of the eye, and the intrinsic muscles of the larynx. Mentioned with them are the infrahyoid group and the diaphragm.

A review of the published work on this subject, carried out very largely by the use of the excellent bibliographies provided by Hines (1927 and 1930), and Hinsey (1934), Barker (1948), Cooper & Daniel (1949), Tiegs (1953) and Cooper (1953) discloses conflicting evidence given by numerous investigators using a variety of animals, but sometimes even by workers using the same species. However, in the last ten years much work has been undertaken in this subject, and the presence of spindles has been demonstrated in muscle groups in which previously they were considered to be absent. For example, spindles have been found in the facial muscles of man (Kadanoff, 1956) and the rabbit (Bowden & Mahran, 1956); in the tongue muscles of the infant (Cooper, 1953); in the extrinsic muscles of the human eye (Cooper & Daniel, 1949; Merrillees, Sunderland & Hayhow, 1950); in chimpanzee and certain ungulates (Cooper & Daniel, 1949); in the vocalis muscle in man (Goerttler, 1950). Paulsen (1958) describes their presence in 'small numbers' in cricoarytenoideus posterior and cricothyroid muscles of man.

The present work demonstrates the presence of neuromuscular spindles in all muscles of the human larynx, and, contrary to Paulsen's findings, these structures are numerous in both cricothyroid and cricoarytenoideus posterior.

In the face of the above evidence there is no support for the hypothesis put forward by Fulton (1946) that non-weight bearing muscles are devoid of spindles.

It has been suggested that a species difference may account for so many workers reporting absence of spindles in these groups of muscle under discussion, and reference to the literature shows that this explanation may be valid, especially in

the case of the rabbit, monkey and domestic animals. Further, it is suggested that the varying reports made by workers using the same animal might, in part, be explained by the fact that all workers have not used serial sections of the whole muscle. Cooper (1953) has called attention to the unequal distribution of muscle spindles both in the extrinsic ocular muscles, and in the infant's tongue. Thus it follows that examination of a block of musculature taken at random might give misleading negative results.

The work of Fernand & Young (1951) on the sizes of fibre components of muscle nerves in the rabbit, and the conclusions they draw from the evidence raise questions of interest relating to the presence or absence of spindles. Their work discloses two types of muscle nerves. One group, comprising the nerves supplying the limb and extrinsic eye muscles, exhibits a bi-modal distribution and contains many large fibres. The other group consisting of nerves supplying muscles of the face, larynx and diaphragm, is composed of fibres less than 10μ in diameter and of uni-modal distribution. Fernand & Young (1951) suggest that these groups of muscles (face, larynx, diaphragm), which are simple in action and non-weight bearing, have little or no proprioceptive supply, and thus would not exhibit spindles. It does not follow, however, that spindles are always to be found in muscles supplied by nerve fibres of large calibre.

For example, though Fernand & Young (1951) have found that the nerves supplying the extrinsic eye muscles of the rabbit contained fibres ranging from $1–20\mu$, with a bi-modal size-frequency distribution, Cooper & Daniel (1949) report absence of spindles in these muscles in the same species. The absence of muscle spindles does not necessarily imply that the muscles in question have little or no proprioceptive supply, for Cooper, Daniel & Whitteridge (1955), using electro-physiological methods, report that the extrinsic ocular muscles in the cat, in which they found no spindles, nevertheless carry afferent impulses. These workers suggest that such muscles are served by some other types of low threshold stretch receptors.

Little work has been done regarding the size and size-frequency disposition of the nerve fibres supplying the human laryngeal muscles. Faaborg-Andersen (1957) suggests there is present a modified bi-modal distribution; and Murtagh & Campbell (1951) show histograms presenting evidence of a bi-modal size-frequency distribution but it must be noted that in this latter case the histograms also show that the majority of the nerve fibres—far from being large in diameter—measure less than $2{\cdot}8\mu$.

Recently in man J. L. Scheuer (1960, personal communication) finds that the size-frequency distribution of the fibres is uni-modal in both recurrent and internal laryngeal nerves, and ranges from $1–18\mu$, the preponderance ($1–8\mu$) being in the internal laryngeal nerve whilst the peak lay between $10–14\mu$ in the recurrent nerve.

The continuation of the present work will be directed towards the determination of the disposition of spindles within the various laryngeal muscles, the source of the afferent and efferent nerve supply of the spindles, and also the distribution and connexion of the laryngeal nerves in the human as seen in serial sections of the foetal larynx.

SUMMARY

1. Neuromuscular spindles have been demonstrated in all the muscles of the human larynx.

2. Certain other types of nerve endings, claw ending, and a spiral ending are recorded.

3. The question as to the significance of the sizes of muscular nerve fibres and of the size-frequency distribution of the fibres is discussed.

The author offers sincere thanks to Mr W. Matthews for the histological preparations, to Mrs P. Thomas and Miss F. Ellis for the production of the microphotographs, and to Professor R. E. M. Bowden for helpful discussions.

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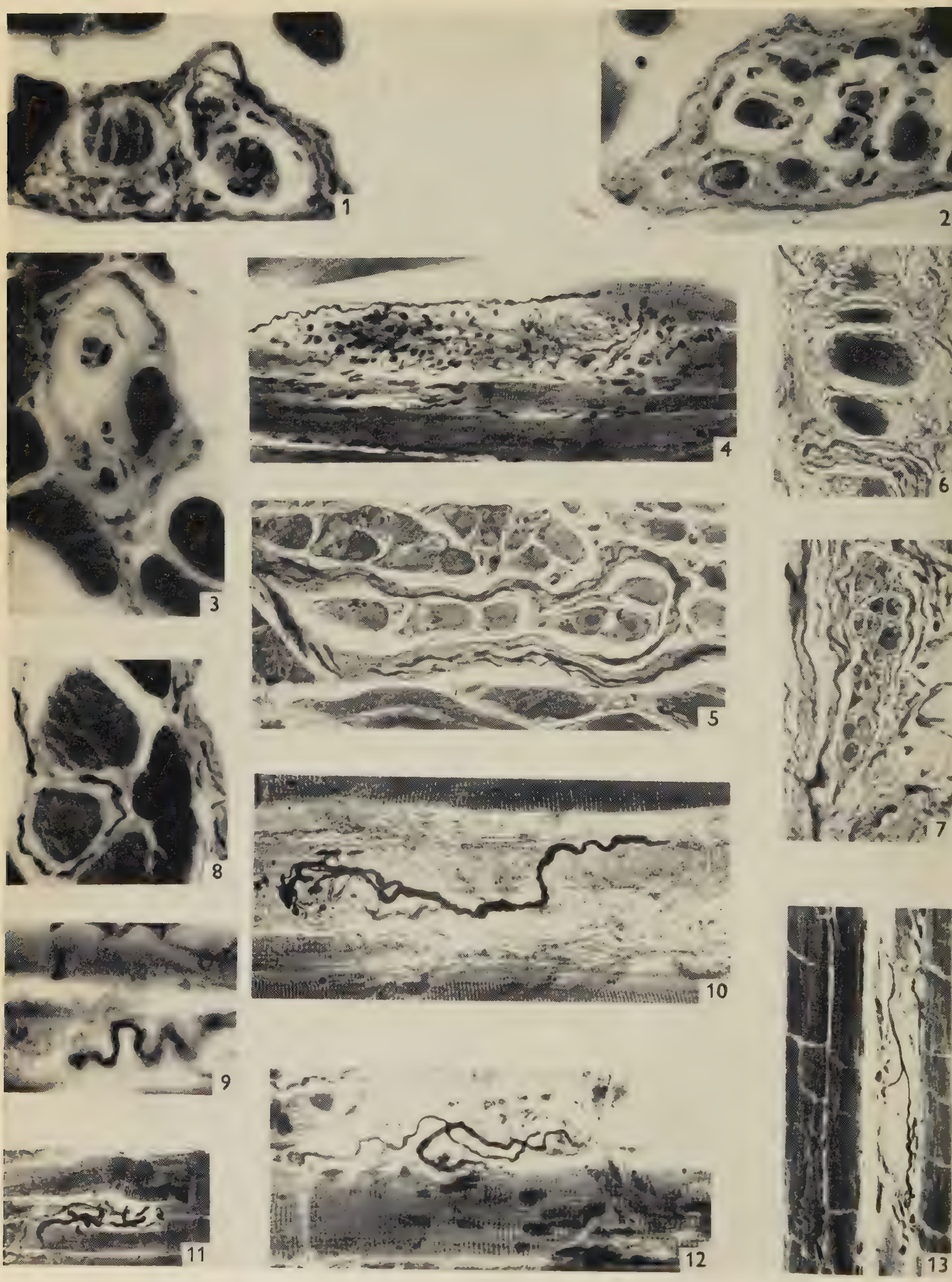

LUCAS KEENE—MUSCLE SPINDLES IN HUMAN LARYNGEAL MUSCLES

(*Facing p.* 29)

EXPLANATION OF PLATE

(All the sections illustrated were stained by the Romanes silver method)

Fig. 1. T.S. of a compound spindle in cricoarytenoideus posterior. ($\times 400$.)
Fig. 2. T.S. of a compound spindle in cricothyroid. ($\times 200$.)
Fig. 3. T.S. of a compound spindle in cricothyroid. ($\times 400$.)
Fig. 4. L.S. of spindle in cricoarytenoideus posterior. ($\times 400$.)
Fig. 5. T.S. of a spindle in cricoarytenoideus lateralis. ($\times 400$.)
Fig. 6. T.S. of a spindle in thyroarytenoideus. ($\times 200$.)
Fig. 7. T.S. of a spindle in arytenoideus. ($\times 200$.)
Fig. 8. A 'claw' nerve ending. ($\times 400$.)
Fig. 9. A spiral nerve ending. ($\times 400$.)
Fig. 10. Motor end-plate. ($\times 400$.)
Fig. 11. Motor end-plate, showing coarse and fine nerve fibres. ($\times 200$.)
Fig. 12. Motor end-plate served by coarse nerve fibre and capillary supplied by a fine fibre. ($\times 400$.)
Fig. 13. A nerve serving two motor end-plates. ($\times 400$.)

THE STRUCTURE AND WEIGHT OF SYNOVIAL FAT PADS

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Several functions have from time to time been ascribed to synovial fat pads. It is of historical interest to recall that Clopton Havers (1691) regarded them as the agents of synovial fluid secretion. The term Haversian glands is still used for these pads but more particularly for the fat pad in the floor of the acetabulum. More modern views as to their function are that they occupy the dead spaces in joints in the various positions, that they serve to ensure that a wedge-shaped interval of suitable dimensions is maintained between the male and female articular surface, thus ensuring effective lubrication (MacConaill, 1950), and that they act as pad oilers (Davies, 1950). Some fat pads have been considered to have more specific functions. Thus the acetabular fat pad may form a soft bed for the ligamentum teres and protect this from damage during movement.

If any of the above-mentioned theories is true, then it could be reasonably expected that these fat pads should differ in their physiological behaviour and possibly in structure from the collections of adipose tissue which occur elsewhere and serve as storehouses of nutritive material. It is often stated that the fat in the subcutaneous tissue of the palms of the hands and soles of the feet is primarily mechanical in function and remains when other fat deposits in the body are depleted (Clark, 1958). Scammon (1919) has investigated the buccal or sucking pad of fat in different states of nutrition. Though it does not differ materially in structure from ordinary subcutaneous adipose tissue, its persistence into adult life is unrelated to the state of nutrition and its degree of development may be asymmetrical. It is well developed in individuals dead of wasting diseases. There is there no apparent relation in the adult between the size of the buccal fat pad and age. Similarly, in the cat, the epidural fat varies in quantity and distribution, but these variations cannot be correlated with the sex, weight, size (as measured by bone length), or nutritional state of the animals (Ramsey, 1959).

The synovial fat pads in human subjects of varying states of nutrition have been studied to determine whether their sizes change in keeping with the general subcutaneous fat stores or remain relatively constant, thus resembling the buccal fat pads.

MATERIAL AND METHODS

Two distinct racial groups, Europeans and Africans, of various ages have been investigated. In the former only the infrapatellar fat pad has been examined whilst in the latter the fat pads from the hip and ankle joints also have been studied. With the exception of the acetabular fat pad these structures are not encapsulated and circumscribed. To obviate errors arising from varying methods and areas of removal all the fat pads from Europeans have been removed by one worker and the African specimens have been obtained locally by another. Records have been made of the

age, sex, height and weight of the individuals and the thickness of the subcutaneous fat in the following positions: immediately to the right of the umbilicus, below the inferior angle of the scapula and at the middle of the back of the upper arm. The thickness of the subcutaneous fat has been measured directly after incising through the skin to the deep fascia. All the fat pads have been weighed and any arthritic changes in the joints noted. The details of the material available are set out in Tables 1 and 2. In addition, specimens from two juvenile European subjects, one newborn and one aged 8 months, and four African juveniles aged 2 months, 8 months, 1½ years and 2 years have been examined.

Table 1. *Data concerning the source of the material, weights of infrapatellar fat pads and thickness of the subcutaneous fat in thirty-one European subjects, together with the mean and standard deviation* (S.D.)

(Arranged in increasing order of subcutaneous umbilical fat thickness.)

No.	Sex	Age	Body weight (kg.)	Body height (cm.)	Weight of intrapatellar pad (g.)	Subcutaneous fat thickness (mm.) Umbilical	Scapular	Brachial	Cause of death
14	M.	63	40	163	14·0	1	1	2	Neoplasm of liver
32	M.	76	41	163	17·2	1	5	4	Sarcoma of humerus
11	M.	65	40	155	21·1	2	3	1	Carcinoma of prostate
19	F.	68	34	142	14·4	3	2	5	Miliary tuberculosis
23	M.	55	65	180	27·0	8	3	3	Renal failure
3	M.	67	62	163	21·7	9	8	9	Lung abscess
5	M.	66	49	173	25·1	9	3	3	Carcinoma of colon
13	M.	70	64	170	21·5	9	6	8	Carcinomatosis
7	M.	57	76	183	26·9	10	9	6	Lymphoma
8	M.	66	50	168	24·1	10	11	8	Cardiac failure
6	M.	59	64	173	22·9	12	8	13	Carcinoma of bronchus
18	F.	57	48	173	18·3	13	6	6	Cardiac failure
16	F.	66	51	160	17·6	15	10	10	Cancer of bile duct
25	M.	76	51	163	19·0	15	5	7	Coronary thrombosis
9	F.	64	69	168	23·3	18	10	15	Malignant peritonitis
10	M.	56	—	165	22·2	18	16	15	Coronary thrombosis
15	M.	61	63	155	22·5	18	8	8	Carcinoma of oesophagus
28	M.	66	80	178	29·1	20	13	10	Cardiac failure
29	F.	80	67	155	19·2	20	10	10	Carcinomatosis
20	F.	58	52	163	16·0	22	8	13	Carcinomatosis
31	M.	72	76	165	27·2	24	14	11	Cardiac failure
24	M.	64	70	178	27·8	25	18	12	Cardiac failure
26	F.	76	76	152	16·5	25	18	10	Abdominal carcinoma
1	M.	60	—	178	22·6	26	10	9	Cerebral haemorrhage
21	M.	55	81	168	22·7	26	9	5	Cardiac failure
22	F.	77	57	157	16·5	32	14	10	Diabetes melitus
12	F.	46	57	163	22·1	35	10	12	Bronchopneumonia
4	M.	56	99	183	22·3	36	15	13	Cardiac failure
17	M.	78	93	180	31·8	38	20	10	Acute pancreatitis
2	M.	62	—	170	28·6	45	13	18	Cardiac failure
27	F.	67	73	173	25·1	59	20	23	Cardiac failure
Mean and S.D.		65 ± 8	62·4 ± 16·1	176·1 ± 9·9	21·9 ± 4·5	18·4 ± 13·2	9·4 ± 5·4	8·8 ± 4·5	

All the synovial fat pads have been fixed in 10 % formalin. Representative blocks of these tissues have been imbedded in paraffin and sections, cut at 8 μ, have been stained with haematoxylin and eosin and with orcein for elastic tissue fibres. Frozen sections have been prepared also and coloured with Sudan red and Sudan black for fat. In all the histological work the technique has been standardized both

with respect to strength of solutions and times. Similar preparations of the subcutaneous fat from the various regions have also been prepared from the European series.

Table 2. *Data concerning the source of the material, weights of fat pads and thickness of subcutaneous fat in twenty-four African subjects together with the means and standard deviations* (S.D.)

No.	Sex	Age	Weight (kg.)	Height (cm.)	Weights of fat pads (g.)						Subcutaneous fat thickness			Cause of death
					Hip		Knee		Ankle		Umbilical	Scapular	Brachial	
					L.	R.	L.	R.	L.	R.				
854	M.	75	57	168	2·4	2·9	6·5	3·5	4·4	1·4	—	—	—	Cardiac failure
855	F.	35	36	155	2·0	2·1	3·0	3·1	2·3	3·5	—	—	—	Tuberculous peritonitis
858	M.	20	20	155	1·1	1·3	5·5	3·4	2·6	2·0	—	—	—	Cardiac failure
868	F.	30	45	160	2·3	2·2	6·6	6·0	3·1	2·8	—	—	—	Carcinoma of vercix
876	M.	—	33	175	2·5	2·8	4·2	7·1	3·7	2·0	—	—	—	?
883	M.	31	38	150	1·6	2·0	4·5	4·6	1·4	1·6	—	—	—	?
890	M.	41	60	163	2·0	1·9	5·1	3·9	1·9	1·9	—	—	—	?
891	M.	16	30	150	1·0	0·9	7·7	7·4	2·3	3·1	—	—	—	Dysentery
897	M.	—	37	150	1·6	1·5	5·3	4·8	1·9	1·8	—	—	—	?
899	M.	17	46	160	1·9	1·6	5·2	6·3	4·6	3·4	—	—	—	Acute pancreatitis
900	F.	17	40	157	1·4	1·4	6·3	4·9	2·1	2·3	—	—	—	Diabetic coma
901	M.	37	38	163	0·9	1·7	6·8	7·4	2·9	3·0	—	—	—	?
903	F.	35	29	157	2·0	2·0	—	—	—	—	—	—	—	Infected ovarian myofibroma
907	M.	25	52	168	1·8	2·3	9·6	6·4	3·6	5·2	—	—	—	Ruptured aneurysm of aorta
920	F.	24	48	155	0·9	0·9	8·2	6·2	1·9	2·0	—	—	—	Uraemia
958	M.	40	49	157	2·2	2·1	10·2	7·4	3·6	3·8	3	6	4	Carcinoma of stomach
987	M.	15	40	165	1·2	1·3	8·3	13·5	2·8	3·1	3	3	3	Sarcoma
1509	M.	35	43	163	3·2	1·9	8·8	9·0	3·9	5·2	3	4	4	Retroperitoneal sarcoma
963	F.	30	28	160	1·4	1·6	3·9	4·4	2·8	2·8	4	3	4	Intestinal obstruction
996	F.	35	43	165	1·4	1·4	8·0	6·4	2·9	2·3	4	5	4	Cardiac failure
957	M.	25	55	178	2·1	2·5	4·7	4·8	3·9	3·7	6	5	8	Mitral stenosis
983	F.	17	34	152	1·7	1·3	7·4	7·6	2·8	3·9	6	3	10	?
980	M.	45	52	173	3·4	2·7	7·2	8·7	3·9	2·9	7	5	5	?
975	F.	30	49	160	1·2	1·6	4·7	3·6	3·0	2·9	17	5	9	?
Mean and S.D.		31 ± 13	41·8 ± 10·0	160·4 ± 7·1	1·8 ± 0·7	1·8 ± 0·6	6·4 ± 1·9	6·0 ± 2·3	3·0 ± 0·8	2·9 ± 1·0	5·9 ± 4·4	4·3 ± 1·1	5·6 ± 2·5	

RESULTS

In the adult, the form, structure and weights of the fat pads are unrelated to the age or sex of the individual or to the presence or absence of arthritic change in the joints. These factors will therefore not be discussed further.

(1) *Structure of the synovial fat pads*

The amount of fatty tissue in the different pads varies, being abundant in those from the knee and ankle but much less in the acetabular pad, which is more fibrous than fatty.

In all cases the fat is divided into lobules by well-developed septa rich in elastic tissue; the elastic fibres run in various directions but predominantly along the lengths of the septa as they are traced towards the synovial surface (Pl. 1, fig. 1). The larger blood vessels lie in the septa and are also richly supplied with elastic tissue and usually show prominent external elastic laminae.

In well-nourished subjects the fat cells within the lobules are closely packed and rounded or polyhedral in shape from mutual compression (Pl. 1, fig. 2). As in subcutaneous tissue, the fat cells contain a single globule of fat and an eccentric

nucleus lying in the pellicle of cytoplasm. No moruloid fat cells are seen. In all cases the fat lobules are separated from the joint cavity by a single layer of synovial cells or an areolar type of synovial membrane.

In subjects exhibiting moderate wasting or undernourishment, as judged by height–weight figures given by Sanderman & Boerner (1949), there is no detectable histological change.

In severe wasting, the total weight of the body being less than 50 kg, there is a detectable reduction in the size of the fat globule; at this stage the fat globule develops a mottled appearance (Pl. 1, fig. 4). The fat cells cease to be spherical and become stellate with branched processes, resembling fibrocytes (Pl. 1, fig. 6), and both the absolute and relative amount of cytoplasm increases. In addition to a single large fat globule there now appears in the cytoplasm a number of smaller fat globules (Pl. 1, fig. 5). In extreme wasting the majority of the cells contain only small scattered fat globules. At this stage blood vessels in the tissue become engorged and prominent, and the cells are separated by conspicuous intercellular spaces (Pl. 1, fig. 6).

In the juvenile specimens, though the fat pads are neither as large nor as heavy as in the adolescents or adults, the structure is similar and the fat cells and globules are only slightly smaller in size than those of the adult (Pl. 1, figs. 2 and 3). In the newborn the vascularity of the articular fat pad with its dilated and engorged capillaries is a prominent feature. The amount of elastic tissue in the septa is much less in the newborn than at later ages. By 2 months of age the amount of elastic tissue has noticeably increased and the individual fibres are thicker. In the $1\frac{1}{2}$-year-old specimen the relative proportion of elastic tissue in the septa and the diameter of the elastic fibres have reached adult proportions.

(2) *Weight of the synovial fat pads*

There is a very significant difference in the mean weights of the African and European subjects. This difference is greater than would reasonably be expected on either genetic or racial grounds, and is therefore probably attributable to differences in the standards of nutrition. In the European series there are nine cases excessive in weight in proportion to height, as judged by the normal standards provided by Sanderman & Boerner. None of the Africans appear to be overweight: the majority, if not all, are probably undernourished. It seems, therefore, that the two series are not comparable and that a separate analysis of fat pad weights has to be performed for Africans and Europeans.

(*a*) *European Series. Infrapatellar pad weight and thickness of subcutaneous tissue*

In all subjects the umbilical fat thickness correlates well with the brachial fat thickness (Pearson's correlation coefficient, $r = 0{\cdot}79$) and with the scapular fat thickness ($r = 0{\cdot}83$). However, the correlation of the umbilical fat thickness with the infrapatellar fat pad weight is poor ($r = 0{\cdot}32$). Thus undernourishment reduces the thickness of subcutaneous fat in all three sites measured, but has considerably less effect on the synovial fat pad. As shown in Table 1, only in extreme emaciation is the synovial fat pad reduced in weight.

The weight of the synovial fat pad is found to bear a closer relation to body height ($r = 0{\cdot}73$) than to body weight ($r = 0{\cdot}65$). This indicates that the fat pad is probably related to skeletal size. On the other hand, as shown by previous workers, the subcutaneous fat thickness correlates much more closely with body weight than with body height.

(*b*) *African Series. Synovial fat pad weight and subcutaneous tissue thickness*

In view of the reported frequent asymmetry in the development and retrogression of the buccal fat pad, the opportunity has been taken to compare three synovial pads on the left and right sides of the body. The acetabular fat pads correlate well ($r = 0{\cdot}82$) but the pads from the knee and ankle seem less symmetrical, probably because of difficulties in removing exactly comparable portions in the two sides.

The remaining data in Table 2 have been subjected to statistical analysis but no very significant results emerge. The only significant correlation found is between the body weight and the scapular fat thickness ($r = 0{\cdot}82$). No significant relation is found between the subcutaneous fat thicknesses at the three sites, nor between the synovial fat pad weights and the body height. The evident reason for these negative results is the emaciation of most of the subjects.

DISCUSSION

It has long been recognized that the fatty tissues vary in their physical properties and structure according to the functions they perform. When storage and packing are the main requirements the fatty tissue is soft, richly supplied with blood vessels and lymphatics and enmeshed in relatively delicate and loose fibrous tissue. This distorts rapidly and easily and regains its form slowly after removal of the distorting agent. Where the fatty tissue is mainly supportive in function as in the palms of the hands, the heels and soles of the feet and over certain bony prominences such as the ischial tuberosity, it is much firmer and its fibrous tissue septa are stronger and rich in elastic tissue. This latter type is appropriately termed adipose-elastic tissue and distorts slowly when subjected to pressure but regains its form quickly when the pressure is removed (Kuhns, 1949). The type found in articular fat pads is clearly adipose-elastic, and doubtless the elastic tissue here as elsewhere in the synovial membrane prevents nipping of the tissue between the articulating surfaces (Davies, 1945). At the same time it will allow movement and distortion to ensure efficient lubrication as suggested by MacConaill (1950). Ramsey draws attention to the heavy septa of loose collagen bundles in fat from the knee joint of the cat, the stroma becoming less fibrous and more delicate towards the joint surface. No mention is made of elastic fibres.

It has often been claimed that the degree of development of adipose-elastic tissue is independent of the state of nutrition of the individual (Batty Shaw, 1901; Wells, 1940; Kuhns, 1949). Scammon (1919) noted a similar lack of relation between the size of the buccal fat pad and the amount of subcutaneous adipose tissue. The relation between nourishment of the individual and size of articular fat pads has been subject to doubt. Stack & Chasten (1949) and Lewin (1952) claim that there is no apparent relation between the infrapatellar fat pad size and the state of

the subcutaneous fat of the individual. Fisk (1952), on the other hand, states that the fat in the synovial membrane shares in the changes which affect the fatty tissues of the body generally and finds no difference in the chemical composition of the synovial fat from that of the subcutaneous tissue. None of these workers, however, advance any figures or measurements in support of their contentions. Furthermore, the fat pads in joints other than the knee never seem to be considered in these claims.

The results of the present study accord with the statement of Stack & Chasten and of Lewin, in that the synovial fat pads are independent of the subcutaneous fat thickness except in extreme emaciation. There is a high and significant correlation between subcutaneous fat thicknesses in the different regions of the body (Edwards, 1960). When, however, the subcutaneous tissue becomes depleted, or nearly depleted of fat, its thickness is in the region of 5 mm. or less; with further wasting the subcutaneous tissues in the various regions do not undergo comparable reductions in the thickness. In the much undernourished subjects in the African series dealt with here, a comparable state of affairs as regards depletion of fat in the articular pads and in the subcutaneous tissue has been reached and the correlation coefficients are low, whilst in the much better nourished European series the reverse is true.

Edwards (1950) estimates that fat virtually disappears from the subcutaneous tissues in the adult of average height when the total weight of the body is 80 lb. or less. At approximately this stage the fat in articular pads begins to waste and the fat cells and their contained fat globules are first seen to decrease in size. In other words, there is a tendency for synovial fat to remain unchanged until that in the subcutaneous tissues and possibly elsewhere has been depleted.

With loss of fat from the synovium there is first a change in the appearance of the fat globule. With further reduction a number of smaller peripherally situated globules of fat appear in the cell; these remain when the central large globule has disappeared. These regressive changes are similar to those described by Batty Shaw and Wells in the subcutaneous fat cells during deposition of fat and are retained in so-called glandular fat. During the early stages of fat depletion from the articular pads there is increased vascularity which may be a factor in fat removal and an alteration in the appearance of the fat globule, possibly indicative of a change in composition. The engorgement of the blood vessels and increased prominence of the tissue spaces, with presumably the presence of more tissue fluid, may indicate a compensatory mechanism to maintain the size of the fat pad. As the fat disappears, not only do the cells change in form but the amount of their cytoplasm appears to be increased.

The form and structure of the synovial fat pads in the newborn are similar to those of the adult, though the fat globules are smaller and the elastic fibres have not fully differentiated. Increase in the size of fat pads during growth must occur mainly by the differentiation of new fat cells. When the African and European series are compared, it would appear that this differentiation of new fat cells after birth may be inhibited in the undernourished and that after a certain stage in postnatal development no new fat cells can be formed. This suggestion reminds one of the findings of Hammond (1950) on the influence of feeding on the pre- and postnatal development of tissues generally.

SUMMARY

1. The structure and behaviour of articular fat pads in different states of nutrition have been studied in thirty-two European and twenty-four African adults and in five juveniles.

2. The articular fat pads consist of elastic-adipose tissue and in the well or moderately well nourished the fat cells are of the usual type seen in fatty tissue elsewhere. In the newborn the cells and fat globules approach adult size but the elastic tissue is not fully developed until about two years of age. In markedly wasted individuals the single large fat globule disappears, the fat cells become stellate and their cytoplasm contains scattered small globules of fat.

3. With wasting, the depletion of the fat from the synovial fat pads does not begin until that in the subcutaneous tissues has been virtually removed; it is accompanied by increased vascularity and enlargement of the intercellular spaces.

4. Except in extreme emaciation, the weights of synovial fat pads are unrelated to the state of nutrition.

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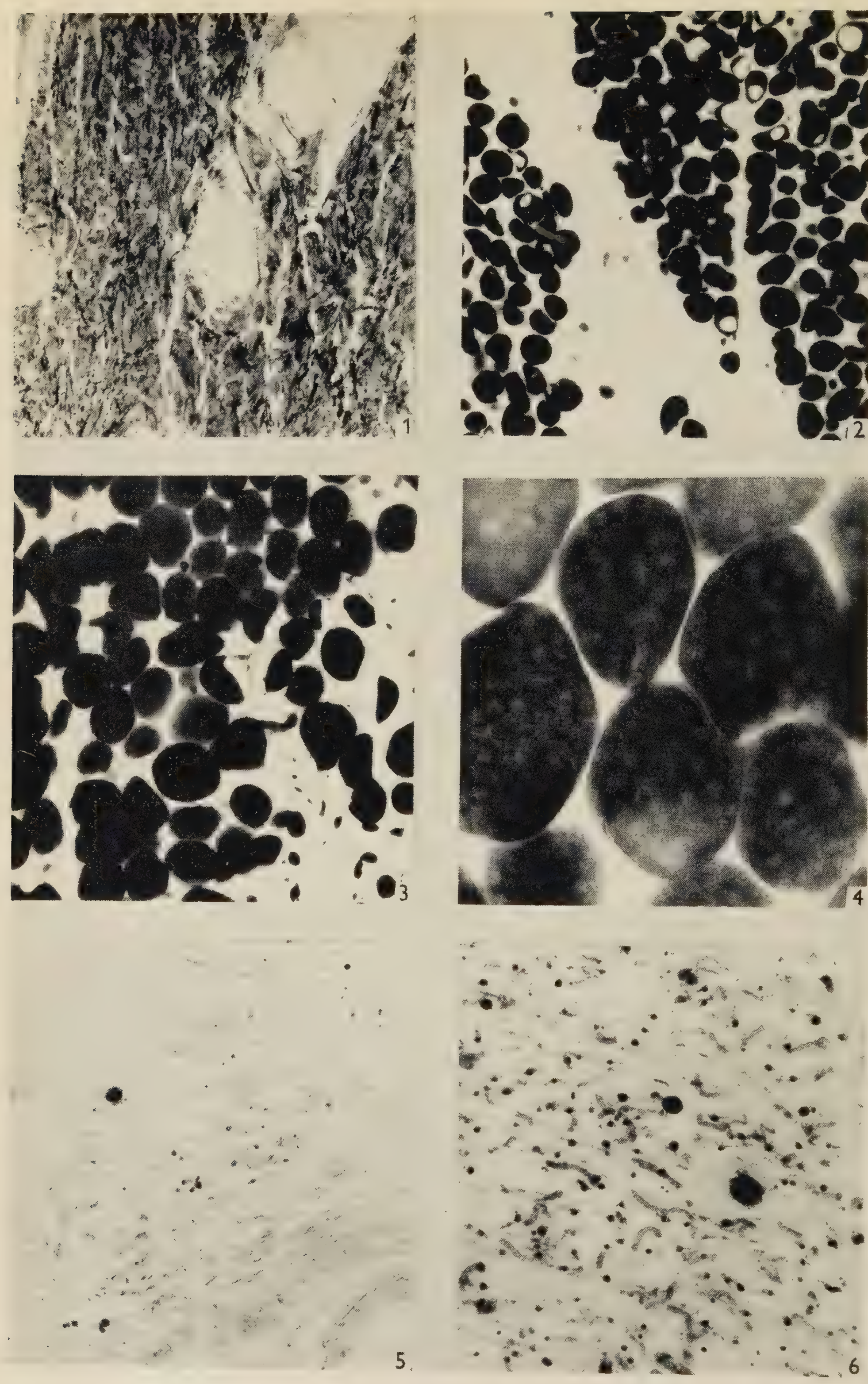

DAVIES AND WHITE—THE STRUCTURE AND WEIGHT OF SYNOVIAL FAT PADS

(*Facing p.* 37)

EXPLANATION OF PLATE

Fig. 1. Section to show the fibrous septa in the infrapatellar fat pad to demonstrate the elastic fibres, most of which are cut transversely. Human aged 1½ years. Coloured with orcein. ×100.

Fig. 2. Frozen section of the infrapatellar fat pad of a human newborn. Coloured with Sudan black. ×100.

Fig. 3. Frozen section of the infrapatellar fat pad of a European male aged 64 years (no. 84). Coloured with Sudan Black. ×100.

Fig. 4. Frozen section of the fat pad from the front of the elbow joint from a severely wasted subject (case 879) to show mottling of the fat globules. Coloured with Sudan black. ×400.

Fig. 5. Frozen section of the infrapatellar fat pad of a severely wasted subject (case 858). Coloured with Sudan black. ×100.

Fig. 6. Frozen section of the infrapatellar fat pad of a severely wasted subject (case 876). Note the scarcity of fat globules and the increased vascularity. Coloured with Sudan black. ×100.

NEURON SIZE AND NEURON POPULATION DENSITY IN THE LUMBOSACRAL REGION OF THE CAT'S SPINAL CORD

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The early investigations on the grey matter of the spinal cord, summarized in the works of Cajal (1911), described the gross arrangement of the cells and illustrated the different types of cells found. Much work since then has correlated the groups of neurons with particular muscles (Balthasar, 1952; Bok, 1928; Elliott, 1944; Romanes, 1951, 1953; Sprague, 1948, 1951, 1958). Rexed (1952, 1954) described lamination in the grey matter of the cord, and Chu (1954) described anterior horn cells which had been isolated from the human cord. The making of models of spinal neurons has been summarized by Haggar & Barr (1950). The use of extracellular and intracellular recording techniques has brought interest back to the individual cell and its neighbours. Rall (1959) summarizes the theoretical calculations using the published spinal cord material, mostly of the perikaryon and the dendrites before their first divisions.

Answers were sought to the following questions:

(1) Can estimates be made of the number, thickness and length of dendrites belonging to spinal neurons?

(2) What is the ratio of dendrite area to perikaryon area?

(3) How does the number of neurons vary in different zones of the grey matter?

(4) Can any estimate be made of the ratio of motor root neurons to intermediate neurons?

MATERIAL AND METHODS

Ten adult cats were anaesthetized with Nembutal supplemented by ether inhalation where necessary.

(1) *Golgi preparations*

The spinal cord was exposed by laminectomy from the cauda equina to the last thoracic root, which was used as a landmark to number the lumbar and sacral spinal roots. Segments of the cord 3–4 mm. long were taken at the levels of the roots. The segments trimmed of the roots and cleared of blood clot were placed on glass wool in labelled tubes containing fixative. Half the segments taken from a cord were stained by the Golgi–Cox method, and the other half by the Golgi–Rapid method. The latter technique resulted in fewer stained cells, but these were usually more satisfactory for measurement, even the finest branches being stained. The axons were also more easily identified. The Golgi–Cox method produced more stained cells in a section but many of the cells were incomplete or badly fragmented. The background, however, was much clearer.

A. *Golgi–Cox method*

This was essentially that described by Sholl (1953).

B. *Golgi–Rapid method* (modified from Sholl, 1953)

This method was much more difficult to standardize, probably due to the uncontrollable osmophilic action of the myelin in the white matter.

(1) The material having been quickly cleared of blood in warm saline was placed on glass wool in a labelled tube containing a freshly prepared mixture of 2·5 % aqueous solution of potassium dichromate (80 ml.) and 1 % aqueous solution of osmic acid (20 ml.). The specimen was left for 4–16 days at room temperature in the dark. The use of ice-cold fixative and fixation in the refrigerator slowed the process but did not markedly improve the results.

(2) The block was removed with a glass spatula, dried on filter-paper, and rinsed in 50 ml. of 0·75 % aqueous silver nitrate. After one further change of silver solution, the block was put on glass wool in 70 ml. of 0·75 % aqueous silver nitrate and left in the dark for 1–6 days.

(3) The block was washed in distilled water with a brush to remove crystals, and then washed for about 6 hr. with changes of distilled water.

(4) Dehydration was accomplished by placing in a stoppered cylinder with 1 part acetone and 2 parts absolute ethyl alcohol (dried with anhydrous copper sulphate) at 37° C. for 1 hr.

(5) Then followed several rinses in dried absolute alcohol, and a quick rinse in equal parts ether and dried absolute ethyl alcohol.

(6) The block was rinsed in 50 % Necoloidine for a few minutes before being left overnight in undiluted Necoloidine.

(7) The specimen was mounted on a fibre block in Necoloidine and left in chloroform for an hour to harden, the fibre block having been previously soaked in an ether–alcohol mixture.

(8) Transverse or longitudinal sections (150–250 μ thick) were cut, mounted serially on slides, quickly blotting with filter-paper. They were coated with a thin solution of Necoloidine, and transferred to 90 % alcohol.

(9) Following a quick passage through dried absolute alcohol the sections were left in terpineol overnight, and mounted in dammar xylol without a coverslip.

(2) *Nissl preparations*

The anaesthetized animals, after ligation of the mesenteric, renal and iliac arteries, were perfused through the aorta with 50–100 ml. of warm normal saline followed by 200–300 ml. of 10 % formol saline. The cord was then exposed, the required segments identified and excised. The left side of the segment was painted with indian ink or the left margin of the segment transfixed with a needle carrying a silk thread. The blocks were further fixed in 10 % formal saline, embedded in wax, sectioned at 10 μ or 15 μ and stained with cresyl violet.

(3) *Weigert preparations of roots*

The ventral roots were identified, fixed, stained and counted as previously described (Aitken, Sharman & Young, 1947).

(4) *Selection of cells*

In the Golgi-stained material, the cells measured were in the ventral horns and the intermediate zones. Occasionally a large neuron was identified in the dorsal horn, but the great majority of neurons in this part of the grey matter were small. Owing to the thinness of the sections compared with the spread of the dendrites, only those branches in the plane of the section were ever fully represented. Though the axon hillock could usually be identified, the axon itself was often cut short, only the thin initial segment being present. It was not possible to differentiate between motor neurons, interneurons and border cells (Cooper & Sherrington, 1940). The measured cells were situated in all parts of the ventral horn and the adjacent intermediate region. Their segmental distribution was: L3, 2 cells; L4, 8 cells; L5, 19 cells; L6, 9 cells; L7, 9 cells; S1, 11 cells.

In the Nissl-stained material, the neurons counted possessed a clear unstained nucleus with a prominent nucleolus and the cytoplasm of the larger cells contained abundant Nissl substance. Occasionally multipolar cells with a nucleus characteristic of a neuron were seen to possess little or no stained Nissl substance. They were situated near other neurons which were fixed and stained satisfactorily. These multipolar cells were considered as neurons and counted if the nucleolus was in the section. Other neurons appeared chromatolytic with an eccentrically placed nucleus and peripheral clumping of the Nissl substance. The presence of apparently degenerating cells was commented upon by Sprague (1951) working on normal monkey material. They appeared amongst otherwise healthy cells. When the nucleolus was present, they were included in the counts. In the dorsal horn an occasional large multipolar neuron was seen, but the majority of cells were small and difficult to differentiate. The numbers of cells in the dorsal horns are probably underestimates.

(5) *Measurements of neuronal size and population density*

A. *Size of perikarya, dendrites and axons*

Measurements were made on the Golgi material, using calibrated ocular graticules and a 4 mm. objective. One small division of the graticule was equivalent to $2{\cdot}5\,\mu$. The following measurements were made.

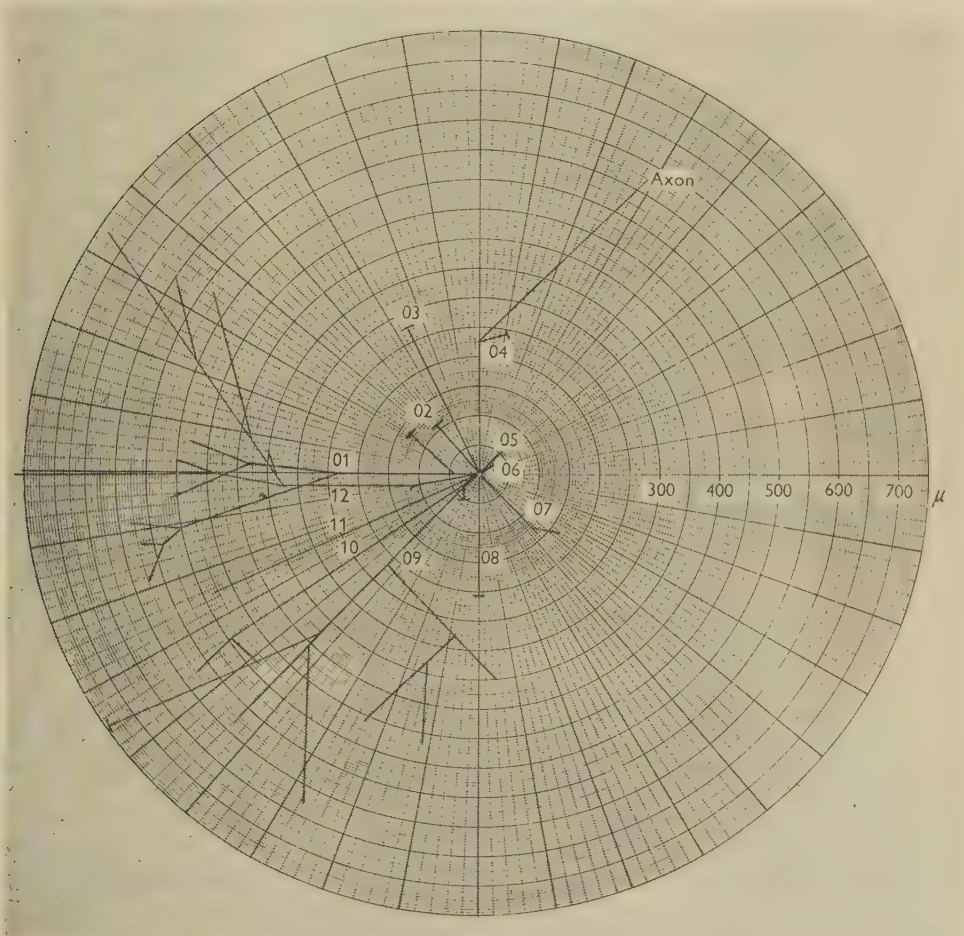
(1) The longest axis of the perikaryon and the maximum breadth at right angles to the long axis.

(2) The branches were numbered and with their subdivisions assessed for length and width (see Sholl, 1953).

(3) The approximate position of origin of the branch on the perikaryon, its direction and the directions of the sub-branches were noted.

The true length of a branch was calculated from the apparent length of the subdivisions, the change in depth of focus between the ends of the subdivision and the refractive index of the glass slide and mountant.

To test the consistency of the results, one of the authors (J.T.A.) remeasured eleven cells previously measured by his co-author and eleven cells previously measured by himself. As far as possible, the same two diameters of each cell were measured on the two occasions. Bridger's assessment of the diameters of a perikaryon were slightly larger than Aitken's, the places where the dendrites joined the



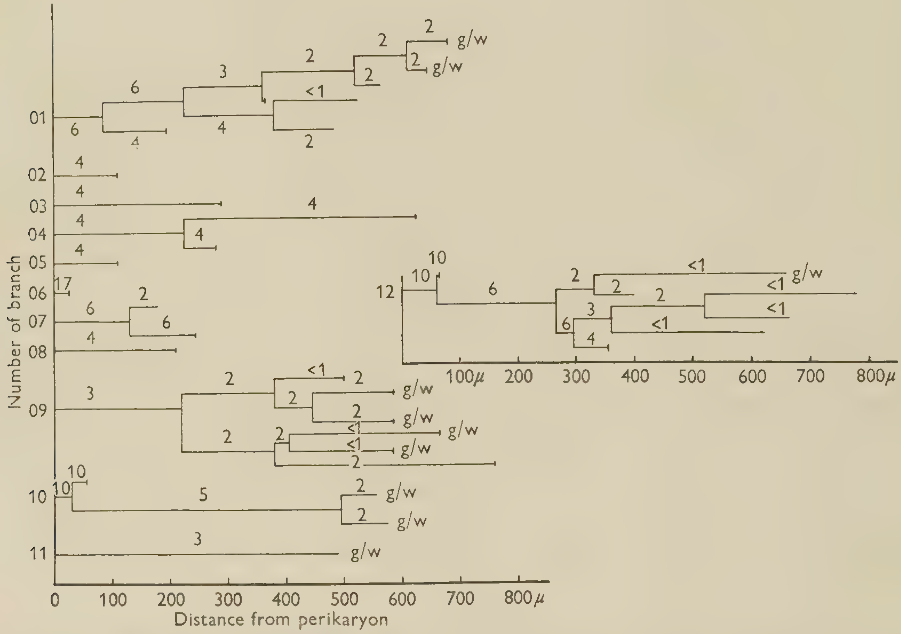
Text-fig. 1. Polar graph of the dendritic pattern of cell no. 16 (see Pl. 1, fig. 2). The central point represents the surface of the perikaryon, the branches are numbered 01 to 12.

perikaryon being impossible to determine accurately. The lengths of the sub-branches of the dendrites were easier to measure consistently. The biggest difference in the perikaryon of a fusiform cell was between 66·5 and 61 μ in the longest diameter.

As between authors, it was found that the mean of the differences between equivalent measurements of cell-bodies significantly exceeded zero ($t = 3{\cdot}38$, $0{\cdot}01 > P > 0{\cdot}001$). The mean differences between equivalent measurements made

by the same author at different times did not significantly exceed zero ($t = 0{\cdot}546$, $0{\cdot}6 > P > 0{\cdot}5$).

No attempt was made to correct the estimates for possible shrinkage. As far as is known, no one has made measurements on the dendrites of unfixed material, though Chu (1954) measured the perikarya of isolated human anterior horn cells. It is possible that shrinkage affects the perikaryon more than the branches. Golgi methods of staining cover the neurons with a thin layer of reduced silver. This 'plating' of the surface will probably affect measurements of the branches more than of the perikaryon.



Text-fig. 2. Diagram showing the length and diameter (in microns) of the branches on the dendrites of cell no. 16. (See Text-fig. 1 and Pl. 1, fig. 2) *g/w*: grey/white boundary.

The dendritic pattern was drawn on polar graph paper, the centre point of the polar graph or a circle of proportional size representing the surface of the perikaryon and the branches radiating out at the appropriate angles and for the corrected lengths (Text-fig. 1). This can only be an approximation as the branches coming off from varying points are projected on to a flat surface. The lengths and diameters of the dendritic branches were also represented as in Text-fig. 2.

The area of each subdivision was calculated, treating each as a cylinder whose mean diameter was known. The surface area of the perikaryon was calculated, treating each as either a sphere, cylinder, cone or double cone according to its shape.

B. *Neuronal population density in the grey matter*

The Nissl material was scanned, using an ocular graticule square with a side 0·36 mm. long. Successive transits were made across the grey matter from medial to

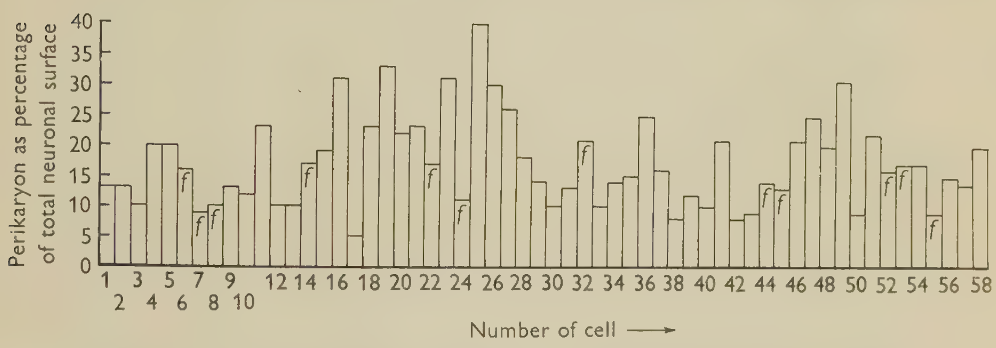
lateral and counts made in adjacent areas. Only neurons with nucleoli were counted. From these figures were calculated the neuronal densities in 100 μ cubes of grey matter. Any neurons in the irregular margin of the grey matter were noted but not used to calculate the densities.

RESULTS

A. *Size of perikarya, dendrites and axons*

(1) *Perikarya*

On the data obtained in this investigation, classification of neurons was not critical as so many intermediate types were found. However, some neurons (with fusiform shapes, Pl. 1, fig. 1) had one diameter of the perikaryon much larger than the other. Their branches (4–9 in number) were usually unevenly divided between the two ends of the cell-body. Occasionally a branch arose from the middle of the



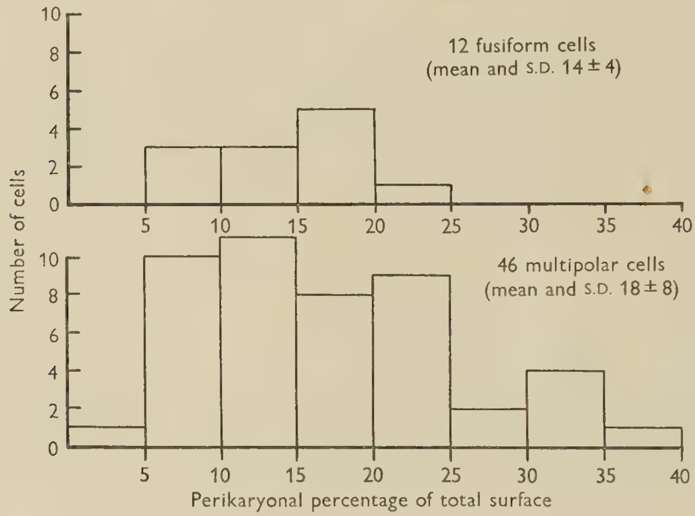
Text-fig. 3. Diagram showing the surface area of the perikaryon as a percentage of the total neuronal area. (*f* = fusiform cell.)

cell-body. Some of the larger fusiform neurons lay near the edge of the grey matter and their branches were orientated along the grey/white boundary. In the twelve fusiform cells measured, the area of the perikaryon varied between about 2000 and 10,000 μ^2. The multipolar cells (Pl. 1, fig. 2) had axes of more equal length and the branches arose from a variety of positions all round the perikaryon. Of the forty-six multipolar cells measured, the largest perikaryonal area measured about 25,000 μ^2 and the smallest about 750 μ^2.

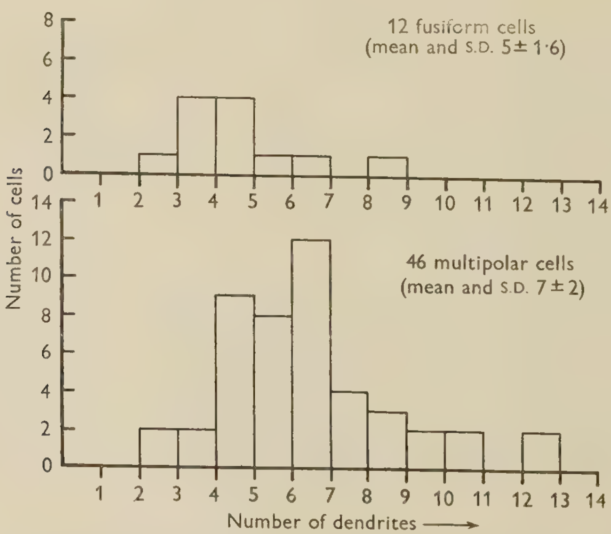
Text-fig. 3 shows the area of the perikaryon as a percentage of the total neuronal surface in the 58 cells. In Text-fig. 4 the cell types have been separated and the numbers of cells having given proportional sizes is indicated. No fusiform cells had a perikaryon contributing more than 25 % to the total neuronal area, whereas seven multipolar cells contributed between 25 and 40 %. One multipolar cell contributed only 5 % of the total. The mean and standard deviation of the perikaryonal areas of these groups are: multipolar cells, 18 % $\pm$ 8 (46); fusiform cells, 14 % $\pm$ 4 (12). These figures emphasize the variability within the groups of cells.

(2) *Dendrites*

The number of dendrites per cell varied considerably in the two groups of cells measured (Text-fig. 5). The mean numbers and standard deviation in each group are: multipolar cells, 7 ± 2 (46); fusiform cells, $5 \pm 1{\cdot}6$ (12). The number of dendrites is probably less significant than the pattern of their branching and their lengths and surface areas. The length and diameter of the dendritic sub-branches varied

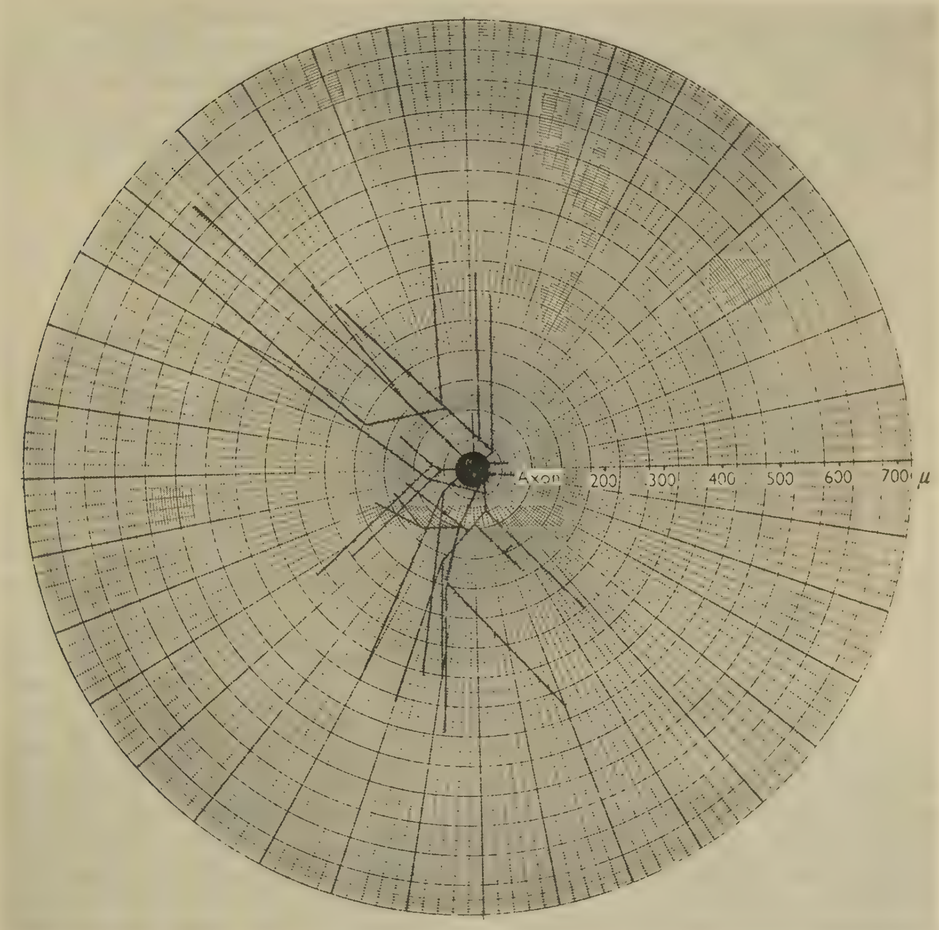


Text-fig. 4. Diagram showing the number of cells contributing varying percentages of perikaryonal surface areas in the two groups of neurons.



Text-fig. 5. Diagrams showing the frequency distribution of the number of dendrites in the two groups of neurons.

considerably and no constant relationship has ever been demonstrated between them. It is thus not possible to estimate the lengths and areas of some of the dendrites, which had obviously been cut short by the planes of section. Some dendrites ended at a distance of over 1000 μ from the perikaryon and others, equally long but tortuous or recurrent, ended nearer the perikaryon (Pl. 1, figs. 3, 4). Some dendrites could be



Text-fig. 6. Polar graph of the dendritic pattern of cell no. 58 (see Pl. 1, fig. 7). The perikaryon is represented by the central circle. The basal dendrites turned towards the apex of the cell and away from the axon.

followed for a distance into the white matter (Pl. 1, figs. 5, 6). The diameter of the dendrites before the first division varied between about 17 and 2 μ. Often it was difficult to decide exactly where the dendrite joined the perikaryon.

Tapering of the dendrites was especially marked near the perikaryon. Branching usually occurred dichotomously (or occasionally trichotomously). The diameters of

the daughter branches were not grossly unequal. Irregular spines could often be seen on the surface of the dendrites (Pl. 1, fig. 6).

The largest total dendritic area calculated for a single cell (no. 20) was 76,270 μ^2. This formed 78 % of the area of the neuron. In this neuron, seven out of the thirteen dendrites appeared to be complete. The others were reduced by various amounts so the whole dendritic area was undoubtedly considerably greater than 76,000 μ^2.

Occasionally the dendrites, instead of forming a uniform pattern around the perikaryon, are grouped and arranged as though orientated in one direction (Pl. 1, fig. 7). In this cell, the axon leaves the base of the pyramid but both basal dendrites turn and ramify towards the apex. This is clearly shown on the polar graph representation (Text-fig. 6).

Though dendrites were traced into the ventral and lateral white matter and in four cells dendrites could be traced into the anterior grey commissure, no dendrites were seen to cross the midline as illustrated by Cajal in foetal and newborn animals.

Recurrent sub-branches of the dendrites could be traced towards the perikaryon or the base of another dendrite (Pl. 1, fig. 4). They usually ended in a spray of fine fibres.

Table 1. *Estimates of the area of dendritic surface outside a radius of 300 μ from the perikaryon*

Cell no.	Total dendritic area	Dendritic area outside 300 μ	Percentage area outside 300 μ
20	76,270	24,852	33
32	25,000	3,291	13
33	20,014	4,500	22·5
54	36,445	15,350	42
56	33,031	6,350	19
57	68,126	21,041	31
58	49,787	12,300	25

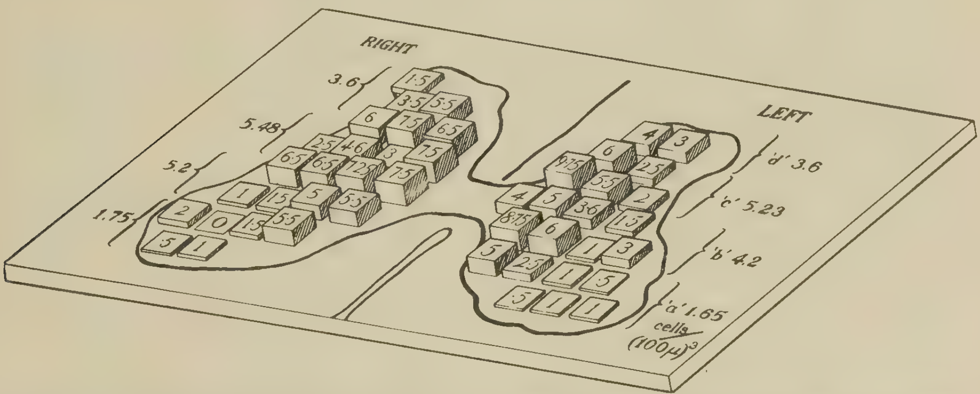
Area of dendrites beyond 300 μ *from perikaryon.* It has been suggested (references summarized in Eccles, 1957) that the effective part of a dendritic surface is within a radius of 300 μ from the perikaryon. Estimates were made on a number of cells of the area outside of this distance (Table 1). In each of this small series of cells a substantial part of the dendritic area was outside a 300 μ radius from the perikaryon, even up to nearly half. It is not known how far out on a dendrite there are boutons, but it is difficult to believe that these large areas are not as intimately concerned with excitation and inhibition as the central parts of the neuron. Electron micrographs show axons in close juxtaposition to dendrites and dendritic spines well below 0·5 μ in diameter in the cerebral cortex (Gray, 1959) and in the spinal cord (Kidd, unpublished). Armstrong & Young (1959), using a post-chroming silver method, have shown small cortical dendrites with boutons for considerable distances from the perikaryon. Whether these are effective synapses awaits proof.

(3) *Axons*

Out of the fifty-eight cells in this series, the axon could be identified in fifty-two. The Golgi–Rapid method stained the finer side branches better than the other method. In four cells, the short portion of axon in the section measured less than

$1\,\mu$ in diameter and these presumably represented only the initial segments. Forty-four axons measured about $2\,\mu$ in diameter, seven were 2–$4\,\mu$ and only one axon measured about $5\,\mu$. Some recurrent branches could be traced back towards the perikaryon (Pl. 1, fig. 4).

As adult cats were used in this investigation, most of the axons emerging from the grey matter were probably myelinated from just beyond the initial segment. Some axons staying within the grey matter may also have been myelinated. The presence of the myelin sheath inhibits the Golgi staining. Where the axon did stain clearly, the cell was probably of the intermediate type and the axon unmyelinated. In the lumbosacral region, the emerging roots run caudally so that the lengths of axons in a transverse section of grey matter would necessarily be short and obliquely running. Greater lengths of axon were measured in the longitudinal sections.



Text-fig. 7. Diagram showing a section in the 6th lumbar segment. Neuronal density is indicated by the height of the column and the figure on top of the column (neurons per $100\,\mu$ cube). The figures along the sides of the grey matter indicate the mean cell density in the four zones measured.

(4) *Neuronal surface area*

The total surface of the perikaryon and the dendrites varied from about 11,000 to $97{,}000\,\mu^2$. The relationship between the total neuronal area and the number of dendrites varied within wide limits. The largest cell (no. 20) also has the largest number of dendrites (13). Dendrites on opposite sides of this cell extended to between 900 and $1000\,\mu$ from the perikaryon, a total spread of nearly 2 mm. Another cell (no. 23) with the same number of dendrites has a perikaryon surface about two-thirds that found on cell 20. One almost complete branch of cell 23 could be traced for about $900\,\mu$ from the perikaryon.

B. *Neuronal density in the grey matter*

From the numbers of cells with nucleoli in each of the fields counted, it was possible to calculate the neuronal density in the fields as cells per $100\,\mu$ cube. Maps similar to Text-fig. 7 were prepared. Not only is there some variation between one section and another but also between corresponding positions on the two sides of one section.

The neurons found in the most ventral and ventrolateral parts of the grey matter are few in number, and mostly of the large multipolar type having a maximum length of about 90 μ and a maximum width of about 60 μ. They are often separated from adjacent neurons by distances of at least 100 μ. Occasionally a large area of grey matter is found with no neurons in it (Text-fig. 7, right side). Comparatively few medium size and small cells are seen except on the medial side of the horn.

The greatest densities of neurons are found in the intermediate zone lateral to the central canal and in the base of the dorsal horns. Frequently the density is seven or eight cells per 100 μ cube and occasionally eleven or twelve cells. These cells are usually much smaller and are packed tightly together. The region round the central canal is usually free of neurons but occasionally a large cell was seen. In the gelatinous substance a few large marginal neurons (Cajal) were identified but most of the cells appeared to have a small amount of cytoplasm round the nucleus and differentiation from neuroglial cells was difficult.

To obtain some measure of the varying cell density, the grey matter was divided into four zones (Text-fig. 7*a*–*d*) passing from ventral to dorsal. Each zone was 0·72 mm. wide and the mean cell density was calculated from the total number of cells observed in the measured fields of the zone.

Counts and calculations made on sixteen sections in this segment (lumbar 6) of the cord showed that the mean neuronal density/100 μ cube in zone '*a*' was 1·6 ± 0·33 S.D. on the right side and 1·65 ± 0·41 S.D. on the left side. Table 2 shows the results obtained when segments L6, L7, S1 of two cats are compared.

This arbitrary division of the grey matter into four zones masked the effects of 'nuclei' or 'columns' in which obviously the neuron density was higher than in the surrounding parts. When an individual neuron is examined it is not easy sometimes to determine to which of the 'nuclei' it belongs. The increased neuronal density in the sacral segments was due to the larger number of medium size and small cells in the sections.

Table 2. *Neuronal density in comparable zones of grey matter in segments L6, L7 and S1 in two cats (see Text-fig. 7).*

The means and standard deviations are based on counts on the number of sections shown in brackets under right zone *a*, unless otherwise indicated.

Segment	Cat	Right, zone '*a*'	Left, zone '*a*'	Right, zone '*b*'	Left, zone '*b*'
L6	1004	1·18 ± 0·36 (9)	1·3 ± 0·31	3·83 ± 0·7	4·0 ± 0·87
L6	1069	1·6 ± 0·33 (16)	1·65 ± 0·41 (15)	3·65 ± 0·77	3·85 ± 0·36
L7	1004	1·3 ± 0·4 (11)	1·65 ± 0·41	3·9 ± 0·63	4·1 ± 0·76
L7	1069	1·6 ± 0·64 (14)	1·5 ± 0·43	3·9 ± 0·95	4·2 ± 0·98
S1	1004	4·0 ± 1·62 (13)	3·4 ± 1·75	5·8 ± 2·9	6·2 ± 2·4
S1	1069	4·2 ± 1·1 (13)	3·2 ± 1·5	6·1 ± 1·4	6·3 ± 0·9

Segment	Cat	Right, zone '*c*'	Left, zone '*c*'	Right, zone '*d*'	Left, zone '*d*'
L6	1004	5·6 ± 1·2	5·8 ± 0·82	3·6 ± 1·3	3·9 ± 1·1
L6	1069	5·8 ± 0·71	5·7 ± 0·57	2·9 ± 1·25	2·9 ± 0·93
L7	1004	6·1 ± 1·1	6·3 ± 0·88	3·5 ± 0·9	4·2 ± 1·3
L7	1069	5·4 ± 1·01	6·2 ± 0·65	2·9 ± 0·75	3·1 ± 0·88
S1	1004	7·5 ± 1·9	9·1 ± 1·9	4·6 ± 0·46	3·65 ± 1·1 (10)
S1	1069	6·4 ± 2·7	6·5 ± 2·5	2·4 ± 2·2	1·6 ± 1·4

C. *Comparison of the numbers of myelinated fibres in the left 6th lumbar ventral root with the neurons in this segment of the cord* (*cat* 1069)

The left ventral root of L6 segment consisted of nine fascicles. Of these the largest was too unevenly fixed to allow of any valuable assessment of the fibre sizes, but the number of myelinated fibres in the fascicle was 1573. The numbers in the other eight fascicles varied between 119 and 671. The root total was 3948 myelinated fibres. 700 fibres less than 8 μ in diameter and 333 more than 22 μ were counted out of the 2375 fibres in the eight smaller fascicles. The largest fascicle also contained a considerable number of these big fibres. The distribution of fibre sizes in the fascicles was sharply bimodal. The peaks were usually about the 6–8 and 20–22 μ groups. The trough was around the 12–14 μ group, two fascicles having no fibres in this group.

The segment of the cord from which the rootlets of the 6th lumbar nerve were judged to emerge was cut into 350 sections, each 15 μ thick. Assuming a regular attachment of the fibres to the segment, then each 15 μ section will have about eleven fibres attached to it. The number of nerve cells in the most ventral zone is about twenty in each 15 μ section, in the whole ventral horn (zones '*a*' and '*b*') is about 85 and if the base of the dorsal horn is also included, as the region of termination of the corticospinal tract, then the figure is about 160 cells. This gives ratios of nerve cells to ventral root fibres of approximately:

2 to 1	Zone '*a*'
8 to 1	Zones '*a*' and '*b*'
14 to 1	Zones '*a*' and '*b*' and '*c*'

From previous work by Romanes (1951) on the cat and by Sprague (1948, 1951) on the Macaque, it is probable that most of the cells of origin of the root fibres are situated in the ventral horn and are not found dorsal to the central canal. Some root fibres are known to arise in cells situated in adjacent segments of the cord and in the ventral horn of the opposite side (Sprague, 1951) so that the ratios of cells to fibres in any one segment are almost bound to be only approximate underestimates.

So far it has not been possible to separate anatomically and accurately the small nerve fibres going to the intrafusal muscle fibres from the larger nerve fibres passing to the extrafusal muscle fibres. It is probable that about a third of the fibres (mostly the small ones under 8 μ) go to the intrafusal fibres (Eccles & Sherrington, 1930; Kuffler, Hunt & Quilliam, 1951). In the specimen examined (L6 left) the total number of fibres counted was 3948. The small fibre components (33 %) would be about 1316 leaving the remainder as motor fibres to extrafusal muscle fibres (2632).

Sprague (1951) was able by means of surgical section of ventral roots and spinal cord of the monkey to produce selective retrograde chromatolysis of spinal neurons. He estimated the percentage of the small neurons (Sprague type II, assumed to be associated with the small fibre system, 10 %). The larger neurons (Sprague type I) were classified as propriospinal or interneuron 52 % of the remainder, border cells 2 % and motor cells 46 %.

The ventral zone of the ventral horn of L6 (left) contains about 20 neurons/15 μ section, making a total of about 7000 neurons in the 350 sections of the segment.

Applying Sprague's percentages to the data now obtained from the 6th lumbar segment, the distribution of neuron types would be:

Small cells (type II)	(to intrafusal muscle fibres 10 %)	700 cells
Large cells (type I)	propriospinal (52 % of remaining 6300)	3276 cells
	border (2 % of 6300)	126 cells
	motor (46 %)	2898 cells

The number of motor cells (2898) approximates to that for the large fibres in the ventral root (2632). Sprague (1951) found that his extrafusal motor cells (type I) were placed around the ventral part of the ventral horn. In the present study also, most of the larger cells were found in the ventral zone. The possible number of small cells (700) is smaller than the number of small fibres in the ventral root (1316). In view of the sparsity of small cells in the ventral zone of the horn, it is probable that most of the cell-bodies of the small fibre system lie further dorsally and, even as Sprague indicated, on the opposite side.

Holmes & Davenport (1940) give the fibre population of the 6th lumbar ventral root in the cat as 5900 myelinated fibres and 290 unmyelinated fibres. The difference between the number in the present study may be partly accounted for by different histological methods (Holmes & Davenport used a silver method) and possibly also by the varying contribution of the segmental nerves to the lumbosacral plexus (Romanes, 1951).

DISCUSSION

Golgi-stained material is the most satisfactory at present available for the investigation of the size and shape of individual neurons. Unfortunately, it is not possible on normal adult material to identify accurately the different functional types of neurons. In the ventral horn of the grey matter there are at present known to be the classical motor neurons, small motor neurons, propriospinal (interneurons), and border cells. The interneurons presumably include 'Renshaw' cells (Renshaw, 1941).

Sprague (1951) using Nissl and silver preparations classified the neurons of the ventral horn of the monkey into two main types. Type I with cell diameters of 25–70 μ he regarded as including large motor neurons, border cells and some interneurons. Type II cells were oval in shape and had long diameters of 15–25 μ. Into this group he placed the small motor neurons and some interneurons. The cells in the present series were also classified according to their shape (multipolar and fusiform). The surface area of the perikaryon varied from 750 to 25,000 μ^2 and there was no obvious difference between the proportions of cells with large and small areas in the two groups. The cells with the larger perikaryonal surface area were nearly all multipolar and were regarded as including large motor neurons, border cells and large interneurons. The smaller cells were of fusiform and small multipolar types. In this group the small motor neurons and interneurons were included.

The surface area of the perikaryon represented about 18 % of the total neuronal surface in the multipolar cells and about 14 % in the fusiform cells (Text-fig. 4). This compares with 10 % of total neuronal surface area found by Sholl (1953) in the cortex of the cat. In the cord, owing to the spread of the dendrites out of the section, figures for the proportion of perikaryonal surface to total neuronal surface will tend

to be large. Estimates given for the total surface area (Wyckoff & Young, 1956; Eccles, 1957) have varied between 5000 and 10,000 μ^2. These numbers are low for the large multipolar cells. In the present series the surface areas of some of the large multipolar cells were in the order of 60,000–100,000 μ^2. The population density of the spinal neural end-feet on the surface of the cell-body and the proximal part of the dendrites has been estimated at about 20 per 100 μ^2 (Wyckoff & Young, 1956). Assuming an even distribution of end-feet over the perikaryon and dendrites, the number would be about 16,000 for large multipolar cells with a surface area of about 80,000 μ^2. Electron microscope studies (Gray, 1959) indicate that some of the boutons present on cortical dendrites are smaller than any that can be seen using stained material, so the number of end-feet may be very much larger. It is not known whether the boutons are evenly distributed or whether they are present on all the finer dendrites. Eccles (1957) suggested that approximate values for the substantially active surface area of the motor neuron may be obtained by neglecting those parts of the dendrites beyond 300 μ from the perikaryon. In the present series many dendrites spread for at least 700 μ and some extended for over 1000 μ from the perikaryon. It is difficult to believe that beyond 300 μ, nearly half of the total receptive surface (Table 1), can be disregarded when considering the activity of the cell.

The conventional description of the arrangement of the cells into nuclei or columns takes account only of the perikaryon and its immediate relations. From the functional point of view it might be more profitable to be able to describe the distribution and volume of the dendritic tree. The position of the perikaryon may be more dependent on the point of egress of the axon than on its source or sources of specific afferents, even though the latter may determine the form and size of the dendrites.

One source of afferent fibres, probably not direct, to the motor neurons is the corticospinal tract. Chambers & Lui (1957) showed in the cat that most of these fibres end in the base of the dorsal horn and the intermediate zone (the intermediate nucleus) of the cord. In this region is a high density of medium and small neurons (Text-fig. 7) many of which are probably intercalated between the corticospinal tract and the motor neurons. The evidence for direct connexions between some fibres of the corticospinal tract and the motor neurons can be explained by a few corticospinal fibres passing into the ventral horn and also by dendritic spread from the motor neurons into the intermediate and dorsal zones. Frank & Sprague (1959) have suggested that the dendrites of some motor neurons can be divided into at least three groups. Those directed dorsally synapse with fibres coming from the ipsilateral dorsal root, those directed medially with contralateral fibres and those directed laterally with fibres from the lateral white columns. The dendrites in the present investigation could not be so clearly grouped though many were clearly orientated (Text-fig. 6 and Pl. 1, fig. 7). The interneurons with widespread dendrites (Chambers & Lui, 1957; Frank & Sprague, 1959) make it difficult to assess the significance of the direction of the dendrites of a cell as a guide to its connexions.

It was not possible in the adult to estimate directly the probable neuronal connectivity of any individual cell owing to the great variability in neuronal density and in dendritic spread.

In the ventral zone of the ventral horn, the neurons are often widely separated, even in the columns or nuclei and it would appear that about half the cells present are probably inter-neurons of some kind. In the whole ventral horn, there are probably at least seven interneurons to one motorneuron and if the intermediate zone is included, there are about thirteen interneurons to each motor neuron.

SUMMARY

1. Segments of the lumbosacral region of the cat's spinal cord were stained by Golgi and Nissl methods.

2. Perikarya measured up to 25,000 μ^2 in surface area and had up to thirteen dendrites with a surface area of about 76,000 μ^2. The dendritic area may form about 80 % of the total receptive area of the neuron.

3. Dendrites could be traced for about 1000 μ from the perikaryon and in some cases nearly half the receptive surface was beyond 300 μ from the perikaryon.

4. The axons of the larger motor neurons were difficult to stain and ran very obliquely through the sections. The largest axon in the grey matter was 5 μ in diameter. Axons in the ventral root of the 6th lumbar segment measured up to 20 μ in diameter.

5. Neurons were most numerous in the intermediate region of the grey matter (7/100 μ cube) and were medium or small in size. The largest neurons were in the ventral zone of the ventral horn where the cells were few in number (1–2/100 μ cube).

6. In the ventral zone of the ventral horn, half the cells are probably interneurons and half are motor neurons. In the whole ventral horn there are about seven interneurons to each motor neuron and if the intermediate zone and root of the dorsal horn are included, then there are probably about thirteen interneurons to each motor neuron.

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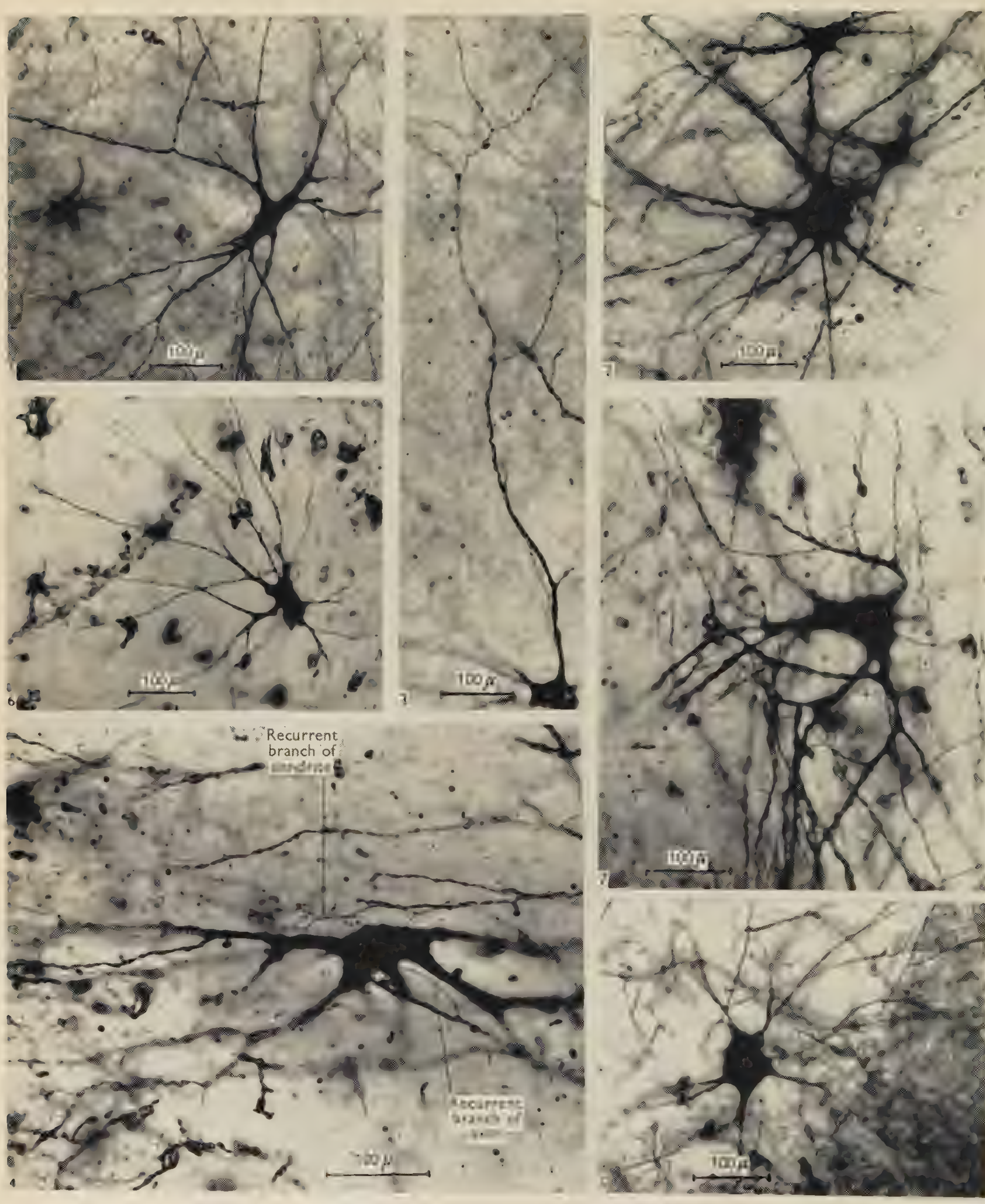

AITKEN AND BRIDGER—NEURON SIZE AND POPULATION DENSITY IN THE LUMBOSACRAL REGION

(*Facing p.* 53)

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EXPLANATION OF PLATE

Fig. 1. Neuron no. 22, fusiform type of perikaryon. (Golgi–Rapid stain.)

Fig. 2. Neuron no. 16, multipolar type of perikaryon. (Golgi–Rapid stain.) Spines can be seen on the dendrites which are in focus.

Fig. 3. Neuron no. 52. One of the dendrites can be followed across the grey matter for nearly 1 mm. (Golgi–Rapid stain, cut longitudinally.)

Fig. 4. Neuron no. 57. Recurrent branches of axon and of dendrite are shown. Connexions with main branches are out of focus but indicated by dots. (Golgi-Rapid stain, cut longitudinally.)

Fig. 5. Neuron no. 26. Axon and some dendrites can be followed into the white matter of the lateral column on r.h.s. (Golgi–Rapid stain.)

Fig. 6. Neuron no. 27, situated along grey/white boundary. Dendrites can be followed into white matter on r.h.s. (Golgi–Cox stain.)

Fig. 7. Neuron no. 58. Basal dendrites turning towards the white matter (l.h.s.). The axon is the lower of the two branches leaving the base of the cell. (Golgi–Rapid stain, cut longitudinally.)

CELL DEATH IN CHICK EMBRYOS AS STUDIED BY ELECTRON MICROSCOPY

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INTRODUCTION

Degenerating cells are found as a regular feature of normal healthy embryos (review by Glücksmann, 1951). I have found them in chick blastoderms studied by light microscopy at least as early as the primitive streak stage (about 18 hr. of incubation), and perhaps even earlier. Their significance at this time is unknown, although in older embryos localized regions of cell death play an important part in the changes in size and shape of developing organs (Glücksmann). The changes occurring in dying cells have frequently been investigated by light microscopy. It has been possible to add to this knowledge in the present study by the use of an electron microscope; for instance, 'banding' together of large granules has been observed in the cytoplasm of degenerating cells.

MATERIALS AND METHODS

Thirty-two blastoderms were examined by electron microscopy. Six of these were from un-incubated eggs, and twenty-six from incubated eggs which had developed as far as the primitive streak or head process stages. Both the area pellucida and area opaca regions were investigated.

Each egg was opened while it was still warm, that is immediately after it was removed from the incubator, in order to avoid post-mortem changes. The fixative was injected into the blastoderm as it lay *in situ* beneath the vitelline membrane and was seen to penetrate immediately into the tissues.

Most specimens were fixed at 4° C. in osmic acid buffered to a pH of 7·2 with sodium veronal (Palade, 1952), and these were usually stained subsequently with a 1 % solution of phosphotungstic acid in the final change of absolute alcohol for 1 hr. Nine blastoderms were fixed in potassium permanganate (Luft, 1956) at a pH of 7·4. Specimens were embedded in the epoxy resin 'Araldite' (Glauert, Rogers & Glauert, 1956; Glauert & Glauert, 1958). Specimens were examined with a Siemen's Elmiskop I*b* electron microscope. Five additional embryos were fixed in Bouin's fluid, serially sectioned at 7 μ and stained either with Feulgen's solution and light green, or with Heidenhain's haematoxylin and eosin.

RESULTS

(1) *Identification and structure of degenerating cells*

To identify the degenerating cells it is necessary to distinguish them from normal (i.e. non-degenerating) cells. In embryos like those of the chick it is also essential to be able to distinguish degenerating cells from certain types of intracellular yolk drops (i.e. the complex yolk drops described by Bellairs, 1958), for after fixation in osmium tetroxide both degenerating cells and yolk drops are highly electron opaque and may contain small granules as well as mitochondria and membranes (Pl. 1, fig. 3; Pl. 3, fig. 9).

The following criteria have been selected as useful guides for identifying degenerating cells. The reasons for regarding them as valid are explained.

(*a*) *Appearance of the nucleus.* It is known from light microscopy that nuclear changes are among the first visible signs of cell death. According to Glücksmann (1951) the initial stage consists in the appearance within the nucleus of certain chromatic patches. Eventually these patches coalesce to form a single 'chromatic cap' sitting on a 'vacuole' formed by the non-chromatic material. Chromatic patches of this type can be seen in both light microscope and electron microscope sections. Pl. 1, fig. 1*a*, is a transverse section across the primitive streak of a chick blastoderm examined by light microscopy (stained with Feulgen and light green). In the centre is a degenerating cell in which dark chromatic patches similar to those described by Glücksmann (1951) lie close to the nuclear membrane. This may be compared with Pl. 1, fig. 1, which is an electron micrograph of a section from the corresponding part of a chick blastoderm of the same age in which similar dark patches lie in the same relationship to the nuclear membrane. It is concluded that since the nuclei shown in both the light photomicrograph and the electron micrograph possess conspicuous chromatic patches, they belong to cells which are in the process of degeneration. The nuclei of the cells shown in Pl. 1, fig. 2, and Pl. 2, fig. 5, possess chromatic caps similar to those described by Glücksmann and are thus also considered to be in process of degeneration.

The dark patches in the nucleus are formed of tightly packed dense granules, each about 75–100 Å. in diameter (see Pl. 1, fig. 4*b*). Frequently they appear to be arranged in short lines like beads on a string.

Unfortunately the thin sections used for electron microscopy do not always pass through the nucleus of a particular cell and it is necessary to have additional criteria for diagnosing degeneration. These criteria can be determined by examining degenerating cells which have already been recognized as such in electron micrographs (because of the appearance of their nuclei, as described above) and looking for other abnormal features in them.

(*b*) *Density of the cytoplasm.* After fixation in osmium tetroxide the cytoplasm of the degenerating cells is denser than that of the surrounding cells (Pl. 1, fig. 2; Pl. 2, figs. 7, 8; Pl. 3, fig. 11). This is so whether or not the tissue has been stained with phosphotungstic acid.

(*c*) *Banding of the cytoplasmic granules.* One of the most characteristic features of degenerating cells is that many of the cytoplasmic granules are aggregated into highly organized and often parallel bands (Pl. 1, figs. 2, 3; Pl. 2, fig. 8; Pl. 3, figs.

11, 12). The appearance of the bands is not always the same, but generally (Pl. 3, figs. 11, 12) each band consists of two or three rows of granules lying parallel to one another. Many of the granules are as much as 500 Å. long by about 250 Å. in width. The long axis of each granule runs along the row and therefore along the band. These large granules are highly electron opaque. The bands may be oriented in several directions in the same cell (Pl. 3, fig. 11), which indicates that banding is not an artefact due to sectioning. In both normal cells and intracellular yolk drops banding of this type has not been seen. It is therefore considered that, when present, banding is indicative of cellular degeneration.

Not all the granules in the degenerating cells are of this type; others are smaller and may be as little as 100 Å. in diameter (i.e. length or width) and are therefore similar in appearance to the granules in normal non-degenerating cells.

Unfortunately, the degenerating cells may be impossible to identify after fixation in potassium permanganate, for after using this fixative cytoplasmic granules cannot be clearly seen in cells (see Pl. 3, fig. 9).

(*d*) *The number of enveloping membranes.* This criterion enables pieces of degenerating cells which have been phagocytosed by other cells to be distinguished from intracellular yolk drops. Each degenerating cell at first possesses its own cell membrane. Thus when a degenerating cell, or part of a degenerating cell, is in process of being phagocytosed it will at first appear to be surrounded by two membranes (each of these being a 75 Å. unit membrane as defined by Robertson, 1957, 1959) although it is likely that they eventually both break down. One of these unit membranes is the invaginated cell membrane of the host, while the other is the cell membrane of the degenerating cell. An intracellular yolk drop is, however, probably never surrounded by more than a single unit membrane (Pl. 1, fig. 3; Pl. 3, fig. 9), which is probably part of the endoplasmic reticulum system. The structure of the membrane around intracellular yolk drops will be discussed more fully in a subsequent paper. Thus, unless membrane breakdown has occurred, two membranes may be expected to surround each phagocytosed degenerating cell, and only one to surround each intracellular yolk drop.

Using this criterion it is now believed that fig. D shown by Bellairs (1958) should be re-interpreted as a degenerating cell and not as a 'complex' yolk drop. This re-interpretation does not invalidate other findings reported in that paper.

Other features of degenerating cells. The following features have also been noted in degenerating cells. They cannot be regarded as diagnostic characters of such cells, however, for they are found in other types of cells too.

Paired membranes of endoplasmic reticulum are present in the degenerating cells. They are usually seen as elongated profiles (Pl. 1, figs. 1, 3) apparently with granules attached to their surfaces and with gaps between paired membranes (Pl. 3, fig. 10). Large circular or oval profiles of endoplasmic reticulum are also present (Pl. 1, fig. 2; Pl. 3, fig. 11).

In specimens fixed with osmium tetroxide and stained with phosphotungstic acid the mitochondria are often more electron opaque than those in the surrounding tissues (Pl. 1, figs. 2, 4*a*; Pl. 2, fig. 6). Furthermore, a light region can often be seen within them (Pl. 1, fig. 4*a*; Pl. 2, fig. 6).

Yolk drops are sometimes present within the degenerating cells (Pl. 3, fig. 10).

They are similar to those present in the cytoplasm of the neighbouring normal cells which have been described previously (Bellairs, 1958). Fat drops comparable with those found in the normal cells (Bellairs, 1958) are often present in the degenerating cells (Pl. 1, fig. 3; Pl. 3, figs. 10, 11).

(2) *Location of the degenerating cells in the embryo*

The anatomical position of the degenerating cells in relation to their neighbours is variable. They may lie between normal cells (Pl. 1, fig. 3) or even between the germ layers (Pl. 1, fig. 1). In some sections they are partially surrounded by a single neighbouring cell (Pl. 1, fig. 2; Pl. 2, fig. 5). It is not, of course, possible without extensive serial sections to be confident that a degenerating cell is surrounded on all sides by an engulfing cell, although by comparison with light microscope studies it seems likely that this is often so (Glücksmann, 1951). When the degenerating cell is apparently surrounded in this way two membranes usually separate the cytoplasm of the two cells. It has been stated above that one of these is the cell membrane of the engulfing cell. This can be seen by inspection of the two membranes at point x in Pl. 3, fig. 10. In some cases the cell membranes are so indistinct that it seems possible that they are in process of breaking down (Pl. 3, fig. 11). The alternative explanation that their indistinctness is an artefact seems less likely, at least for the specimen shown in Pl. 3, fig. 11, for other membranes are clearly visible.

The degenerating cells are present in all parts of the chick blastoderm at the primitive streak and head process stages, both in the area pellucida and area opaca; they seem to be particularly common in the most anterior part of the area pellucida. They have not been seen in the area pellucida of un-incubated blastoderms but are present in the area opaca at that early stage. They are uncommon in the area pellucida after about 48 hr. of incubation.

DISCUSSION

The cells described in this paper have been interpreted as degenerating cells on the basis of a comparison with the findings of light microscopy. Under the light microscope the nuclei of such cells have a characteristic appearance, the chromatic material being aggregated into masses (Leuchtenberger, 1950; Glücksmann, 1951). The electron microscope shows the same phenomenon, the chromatic masses then appearing as collections of small dense particles. (Leuchtenberger has shown by cytochemical means that the development of pyknotic nuclei of this type is accompanied by some loss of protein.) The so-called nuclear 'vacuoles' of light microscopy, however, have not been seen by electron microscopy. It is possible that this vacuolated appearance of light microscopy may be due merely to the fact that some regions of the nucleus are very much less dense than the chromatic mass, and consequently resemble vacuoles when seen under the light microscope.

One of the most conspicuous changes occurring in the cells regarded as degenerating is not visible under light microscopy. This is the occurrence in the cytoplasm of large granules arranged in regular bands. There is at present no evidence to indicate how they are formed. It is possible that they are formed by

the combination of several 100 Å. granules (ribonucleoprotein particles of Palade (1955), and of Palade & Siekevitz (1956)). Since, however, there appears to be a higher density of cytoplasmic granules in the degenerating than in normal cells, it seems possible that some at least of the structures may be derived from the breakdown of other cytoplasmic constituents. It is even possible that there is a loss of water during degeneration which results in the granules becoming more densely packed together.

Light microscope studies have shown that during cell death the cytoplasm also becomes 'vacuolated'. It is possible that the large spaces bounded by membranes (Pl. 1, fig. 2; Pl. 3, fig. 11) correspond with these vacuoles. Similarly, in a study of developing chick neuroblasts, evidence was presented that the 'vacuoles' of light microscopy may correspond with membrane-bounded spaces of electron microscopy (Bellairs, 1959).

There is considerable evidence from light microscope studies that the production of fat drops accompanies cell degeneration (Glücksmann, 1951). Fat drops are usually considered to result from the breakdown of other cell constituents. Fat drops are, however, common throughout the chick blastoderm at this stage.

The association between many enzymes and mitochondria is well established (Gustafson, 1954). No evidence appears to be available to indicate whether or not autolytic enzymes are also associated with mitochondria. Degeneration of the mitochondria themselves, however, probably occurs, for it has been shown that not only are they more electron opaque than those in neighbouring cells but that a lighter area appears within them. This lighter region may perhaps be due to a 'vacuole' appearing within each mitochondrion; alternatively, it is possible that each mitochondrion is bent around a core of cytoplasm.

Fate of the degenerating cells

It is known from studies by light microscopy that dead or dying cells may sometimes be ejected from embryonic tissues (Holtfreter, 1948). The cell seen in Pl. 2, fig. 8, may be in process of ejection. The convergence of the 'banding' toward one end thus might be an expression of the tension effects acting on a cell under these circumstances.

It is also known from light microscopy that degenerating cells may be phagocytosed (Glücksmann, 1951). The ultimate fate of the engulfed cells, or pieces of cells, has not been followed in the present study. They are known from light microscopy to break up into 'degeneration granules' (Glücksmann, 1951). These 'degeneration granules' probably remain as discrete structures for so long as the membrane(s) around them remains intact.

The changes in position which the degenerating cells undergo in relation to their neighbours (for instance, becoming engulfed by them) suggest that physiological changes take place at the cell surface. It is interesting, therefore, that the cell membrane of a degenerating cell often appears to remain intact even when considerable changes have already taken place in the cytoplasm.

There is no evidence to indicate the origin of these particular degenerating cells. From their relative frequency in the anterior border of the area pellucida at the primitive streak and head process stages it was thought possible that they might be

primordial germ cells, for it is in this region that primordial germ cells first appear (Swift, 1914; Simon, 1957). The fact that the degenerating cells are also found in all regions of the area pellucida as well as in the un-incubated blastoderm suggests that many if not all of them have a different origin.

SUMMARY

1. The nuclei of the cells which are considered to be degenerating contain very dense patches which probably correspond with the chromatic patches of light microscopy.

2. The cytoplasm of the degenerating cells is very dense after fixation in osmium tetroxide. It appears to contain more cytoplasmic granules than do the neighbouring normal cells. These granules tend to be larger (about 500 Å. in length) than in normal cells (about 100–150 Å. in diameter) and are aggregated into regularly arranged bands. Abnormal mitochondria and large spaces bounded by endoplasmic reticulum are also described.

3. The cell membrane of a degenerating cell often appears to be intact when considerable changes have already taken place in the cell.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Degenerating cell lying between ectoderm and mesoderm. The section passes through the nucleus. Note: chromatic patches (*c.p.*); membranes of endoplasmic reticulum (*e.r.*); nuclear membrane (*n.m.*). Osmium tetroxide fixation. ×8500.

Fig. 1*a*. Degenerating cell in the ectoderm of the area pellucida (light microscopy). Note: chromatic patches (*c.p.*) in the nucleus; cell wall (*c.w.*). Stained with Feulgen and light green. ×1500.

Fig. 2. Degenerating cell enclosed by another cell. The nucleus of the surrounding cell is visible in the lower right corner (*n'*). The nucleus of the degenerating cell has a cap of electron opaque granules (*n.c.*) (see fig. 4*b*) and the nuclear membrane appears to be in the process of breaking down (arrows). Note: banded granules (*b.g.*); abnormal mitochondria (*a.m.*) (see fig. 4*a*), and large space apparently bounded by a membrane (*sp.*). Osmium tetroxide fixation. ×13,000.

Fig. 3. Degenerating cell lying between two other cells which are situated to the left and right of it. The nucleus of the left cell lies in the lower left corner (*n'*). The left cell contains an intracellular yolk drop, and this contains two large fat drops. Note: banded granules (*b.g.*); single membrane surrounding the intracellular yolk drop (arrowed); fat drops (*f.*). Osmium tetroxide fixation. ×15,000.

Fig. 4. Parts of the cell shown in fig. 2 enlarged.

Fig. 4*a*. Abnormal mitochondria which are extremely dense and possess a lighter region within them. Since this light region contains granules comparable in size to those in the neighbouring cytoplasm, it is possible that it is a core of cytoplasm around which the mitochondrion is wrapped. ×43,500.

Fig. 4*b*. Enlargement to show granules (each about 75–100 Å.) in nuclear cap. ×43,500.

Plate 2

Fig. 5. Degenerating cell surrounded by another cell. Nucleus of the surrounding cell is to the left (*n'*). Note: nucleus of degenerating cell (*n*); and abnormal mitochondria. Osmium tetroxide fixation. ×7400.

Fig. 6. Enlargement of part of section adjacent to fig. 5 to show abnormal mitochondria. ×26,000.

Fig. 7. Degenerating cell lying between two cells which are situated to the right and left of it. Note: two arms extend from the right cell and partially embrace the degenerating cell (arrows). Osmium tetroxide fixation. ×13,000.

Fig. 8. Degenerating cell lying between the mesoderm and the endoderm. Banded granules (*b.g.*) radiate out from the pointed end. Osmium tetroxide fixation. ×18,250.

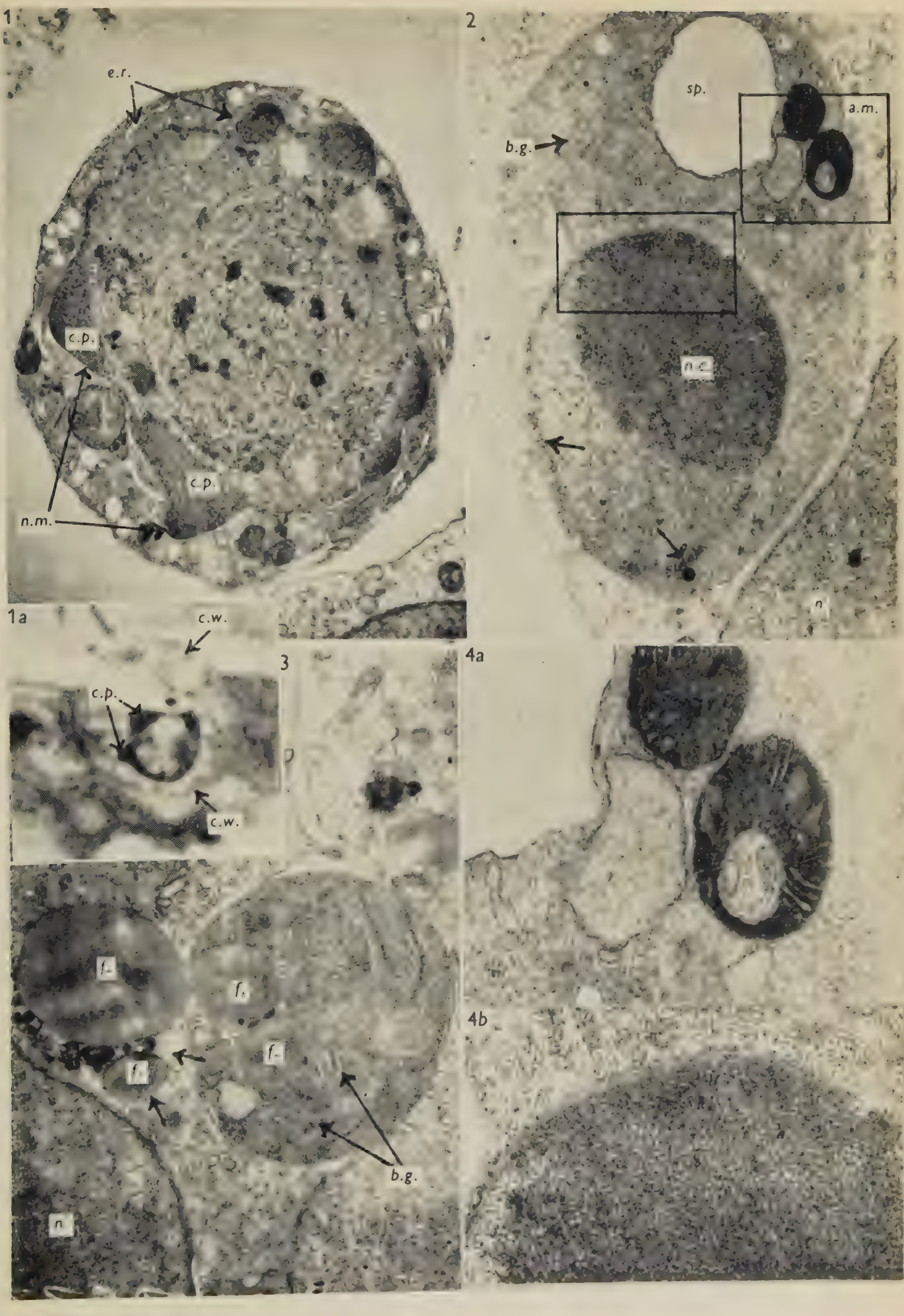
Plate 3

Fig. 9. Two intracellular yolk drops, type A (*A.*) and complex (*C.*) (see Bellairs, 1958). Note: granules can be seen within the yolk drops, but are scarcely visible in the cytoplasm of the cell. Potassium permanganate fixation. ×17,000.

Fig. 10. Degenerating cell surrounded by several normal cells. A type A yolk drop (*A.*) is present in the degenerating cell. Osmium tetroxide fixation. ×14,500.

Fig. 11. Degenerating cell surrounded by another cell. The cell membrane of the degenerating cell appears to be in process of breaking down (arrows). Note: fat drop (*f.*) and banded granules (*b.g.*). Osmium tetroxide fixation. ×25,000.

Fig. 12. Enlargement of the banded granules seen in fig. 11. ×63,000.



RUTH BELLAIRS—Cell death in chick embryos

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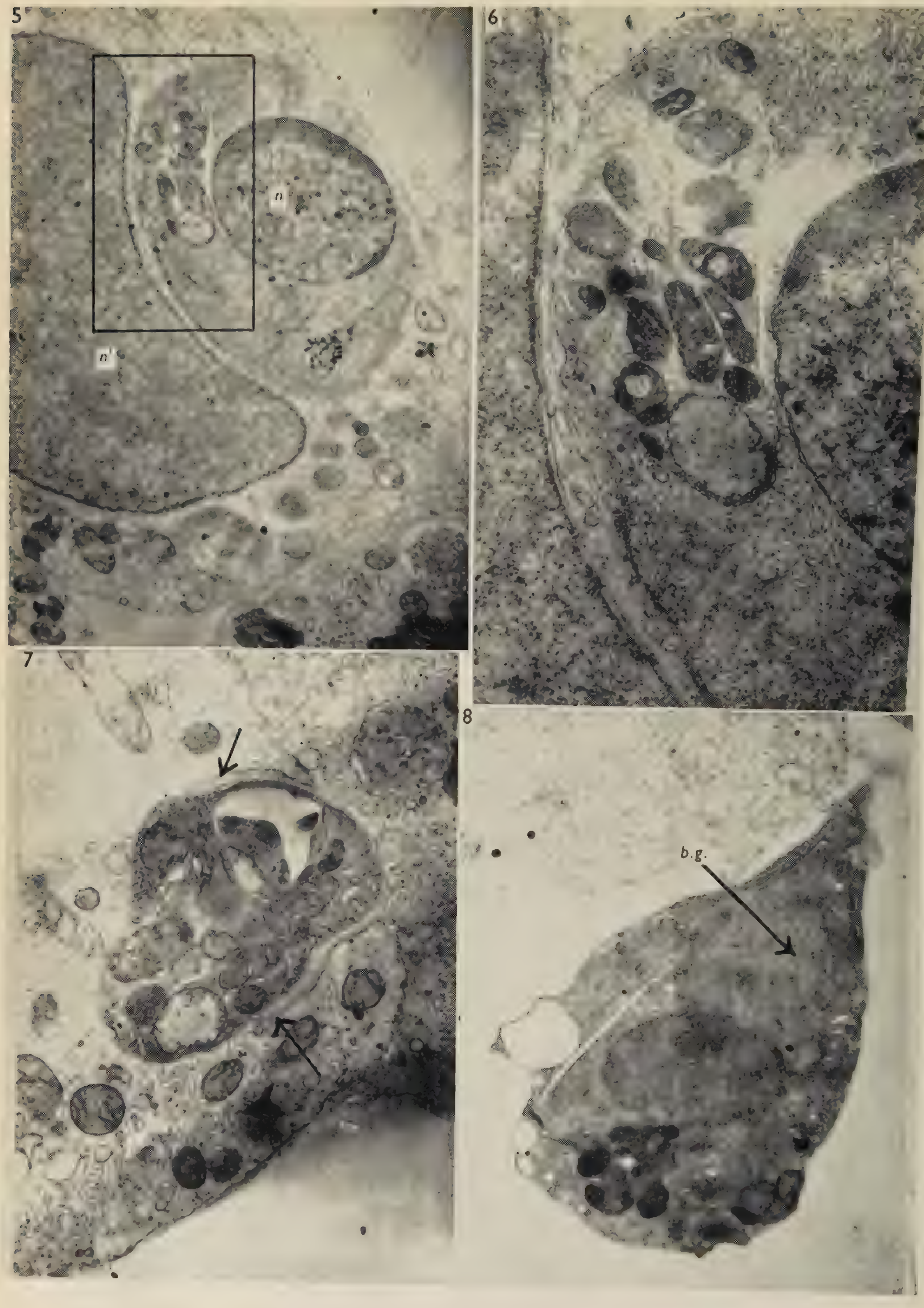
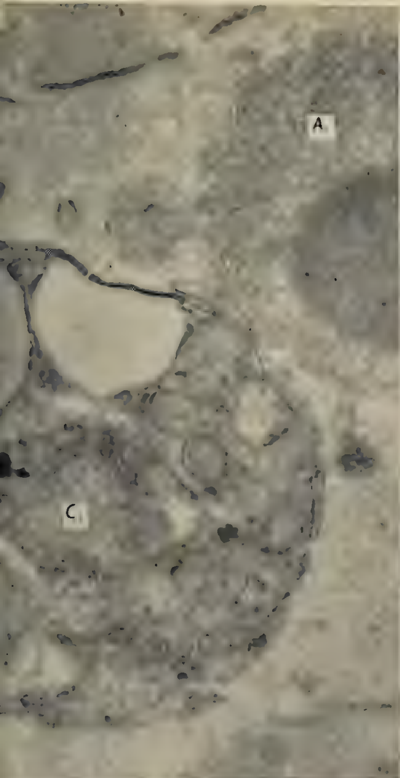
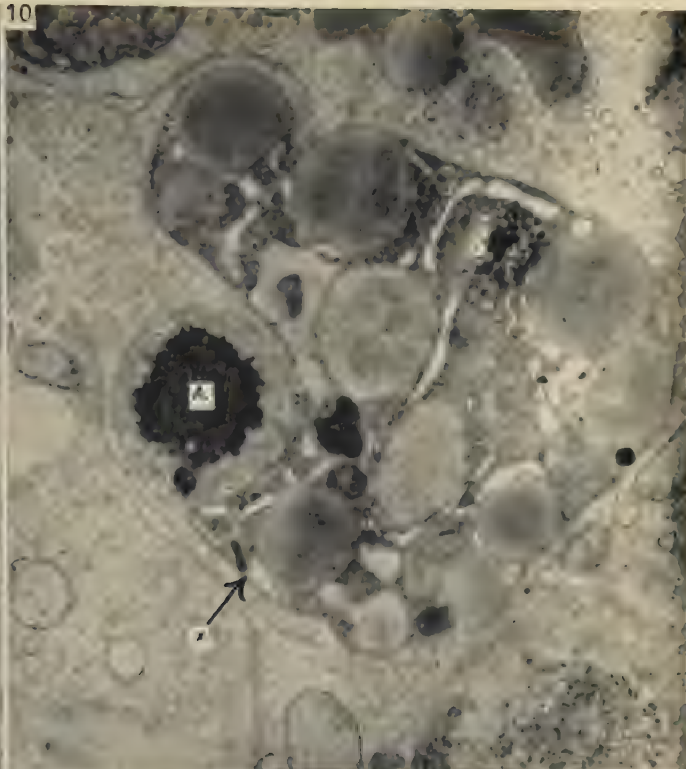


RUTH BELLAIRS—CELL DEATH IN CHICK EMBRYOS





RUTH BELLAIRS—CELL DEATH IN CHICK EMBRYOS

OBSERVATIONS ON THE RETRACTOR CLITORIDIS AND RETRACTOR PENIS MUSCLES OF MAMMALS, WITH SPECIAL REFERENCE TO THE EWE

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INTRODUCTION

Smooth muscle, although generally described as one of the components of a particular organ, occasionally resembles striated muscle in being grouped longitudinally into a separate entity. Striking examples of this peculiarity are to be found in the bilateral retractor penis muscle and its female homologue, which are comparable in form with the adjacent perineal skeletal muscles.

In the male, the retractor penis is usually described as being attached to the ventral surface of the first or second coccygeal vertebra, and continuing in a caudoventral direction between the levator ani muscle and the rectum, to be inserted into the penis towards the end of its free extremity.†

The female homologue is less frequently recorded. It is commnonly known as the retractor clitoridis, but also has been called by such names as, in the mare, the anogenital ligament (Ellenberger & Baum, 1921; M'Fadyean, 1922), the muscular ligament of the vulva (Fleming, 1873), the labial ligament (Habel, 1953); in the cat, the caudovaginalis muscle (Davison, 1923); and the retractor cloacae muscle (Nishi, 1938) in animals with a common urorectal opening. Some authors have stated that the muscle terminates in the clitoris, but others are less definite, saying merely that it goes to the genitals, or that it terminates in the vulva, or that it disappears among the fibres of the constrictor vulvae muscle.

Although the most commonly described origin of the retractor penis is from the first or second coccygeal vertebra, a sacral attachment is occasionally mentioned. In some species, and perhaps also breeds, there is no rectococcygeal part, the muscle arising proximally in these instances in the lateral rectal wall. Such a rectal origin is recorded for the dog (Paulet, 1877; Langley & Anderson, 1895; Bradley, 1948; although a coccygeal origin has also been described—Miller, 1952); according to Paulet (1877), it is the same in wolves, and certain species of deer, although in one species he found a sacral attachment. Mivart (1881) gives a rectal origin for the retractor penis of the cat. In the horse, in which a coccygeal (or sacral) origin is constant, the muscles from each side unite beneath the rectum to form a sling, before

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† Some early anatomists (e.g. Owen, 1868) describe levatores as well as retractores penis muscles in several species, arising from the pubis and serving mainly as compressors of the vena dorsalis penis; levatores clitoridis muscles have been recorded in the female elephant, though the origin was not stated (Paterson & Dun, 1898). Most later writers do not mention two such distinct muscles, however—only retractores.

continuing to the penis or clitoris; as a result, the proximal (rectococcygeal) part of the muscle in the horse has frequently been termed the suspensory ligament of the anus, although its continuation as the rectopenile part has been appreciated. May (1955) described a similar suspensory ligament in the sheep; in the ram, it is continuous with the retractor penis, but in the ewe there is no mention of a retractor clitoridis.

The muscle is always described as long, cylindrical, pale, enclosed in a connective tissue sheath, and composed of smooth muscle. In some species, dissections have shown the main innervation of the retractores muscles to be from a branch of the pudendal nerve (bull—Larson, 1953; sheep—May, 1955). Although the chief innervation may be from the pudendal nerve, and this has been shown experimentally to contain orthosympathetic fibres in certain mammals (cat and dog—Langley & Anderson, 1895; cat—Oppenheimer, 1938; bull—Larson, 1953), the pelvic plexus may also contribute (Langley & Anderson, 1895). There is some indication that sacral para-sympathetic fibres are also involved (Langley & Anderson, 1895; Oppenheimer, 1938), but the precise route taken by such fibres is unknown. No reference can be found concerning its blood supply.

The muscle is present in the horse (males—Owen, 1868; both sexes—Fleming, 1873; Ellenberger & Baum, 1921; M'Fadyean, 1922; Bradley, 1922; Sisson, 1953; Habel, 1953); cattle (males—Owen, 1868; Sisson, 1953; Larson, 1953; females—Geiger, 1956), sheep (males—May, 1955; Geiger, 1956) goat (Geiger, 1956), dog (males—Owen, 1868; Paulet, 1877; Bradley, 1948; Miller, 1952; both sexes—Langley & Anderson, 1895); cat (males—Mivart, 1881; Oppenheimer, 1938; both sexes—Langley & Anderson, 1895; Davison, 1923). The retractor penis has been recorded also, for males only, in the tiger, wolf, three species of deer (Paulet, 1877), pig (Owen, 1868; Sisson, 1953), kangaroo (Owen, 1868; Paterson, 1907), platypus (Owen, 1868), and guinea-pig (Kochakian, Tillotson, Austin, Dougherty, Haag & Coalson, 1956). The retractor penis or clitoridis muscle is apparently absent in the rabbit (Langley & Anderson, 1895; Bensley, 1948), the rat (Greene, 1955) and in man (Nishi, 1938). Nishi states that it is best developed in marsupials, carnivores and animals with well-formed tails, and also commented that there is no homologous structure in reptiles. Paulet (1877), who found no retractor penis in two species of monkey, considered that it occurs only in animals with a penis fixed in the abdominal wall; in primates, which have a free penis, it would be unnecessary from a functional point of view. Paulet examined only males, and did not consider the probable function of the homologous muscle in the female.

Physiological experiments on the dog and cat (Langley & Anderson, 1895; Oppenheimer, 1938) and on the bull (Larson, 1953), suggest that in these species at least, the retractores muscles function as their names imply. Sisson (1953) suggested further that the proximal part of the muscle may act as an accessory anal sphincter in the horse. Habel (1953) considered that in the mare the 'labial ligament' may transmit the pull of the ventral part of the levator ani muscle to the labia, and thus cause gaping of the vulva.

No reference can be found to the embryology of this muscle in any species.

The investigations described in this paper comprise a special study of the retractor clitoridis muscle in the ewe—including its development, microscopical structure and alterations in pregnancy.

The morphology and morphogenesis of the muscle were investigated as part of a comprehensive study of the gross anatomy of the pelvic and perineal region of the ewe (Bassett, 1956) and further observations have been made subsequently on animals killed for other experiments.

The chemical changes during pregnancy in the retractor clitoridis muscle have been the subject of a separate experiment (unpublished) by another investigator, R. J. Newbold. A summary of his findings, communicated privately, is included.

Observations have been made also on the retractor clitoridis and retractor penis muscles in certain other species.

MATERIAL AND METHODS

In all, eleven different genera were investigated.

Sheep

For gross anatomy, 126 ewes were dissected, of three different breeds and one cross-breed, ranging in age from 70 days prenatal to 8 years postnatal; two rams were also included. Some of the specimens were fixed with 10 % formalin, but the majority were dissected fresh.

The early development of the retractor clitoridis muscle in relation to other perineal structures was studied microscopically in a sectioned series of seven embryos and foetuses of unknown breed with crown-rump lengths between 9·5 and 46 mm. Relating crown-rump length to foetal age, it can be estimated that the largest of these was approximately 42 days old (Malan & Curson, 1936; Winters & Feuffel, 1936; Harris, 1937); the smallest embryo was known to be 28 days old. The gonads of the three youngest (9·5–15 mm.) were undifferentiated, and the other four were females. A graphical reconstruction was made from traced projected sections of the oldest (46 mm.) foetus.

The microscopical structure was examined in one complete muscle (approximately 2½ in. long) and on segments from the clitorial termination of the muscles of ewes killed to determine the effects of pregnancy. These tissues were fixed in 10 % neutral formalin, sectioned at 4–6 μ and stained with Weigert's haematoxylin, followed by van Gieson and orcein, haematoxylin-azur II-eosin (Maximow, 1924), toluidine blue, and Long's (1948) silver impregnation method.

The changes in pregnancy were studied in forty-eight ewes at several different stages of gestation including a group of non-pregnant controls (Table 1). The muscles from each side were dissected out and weighed together. A small portion was taken for histology and the remainder used for chemical analysis.

Other species

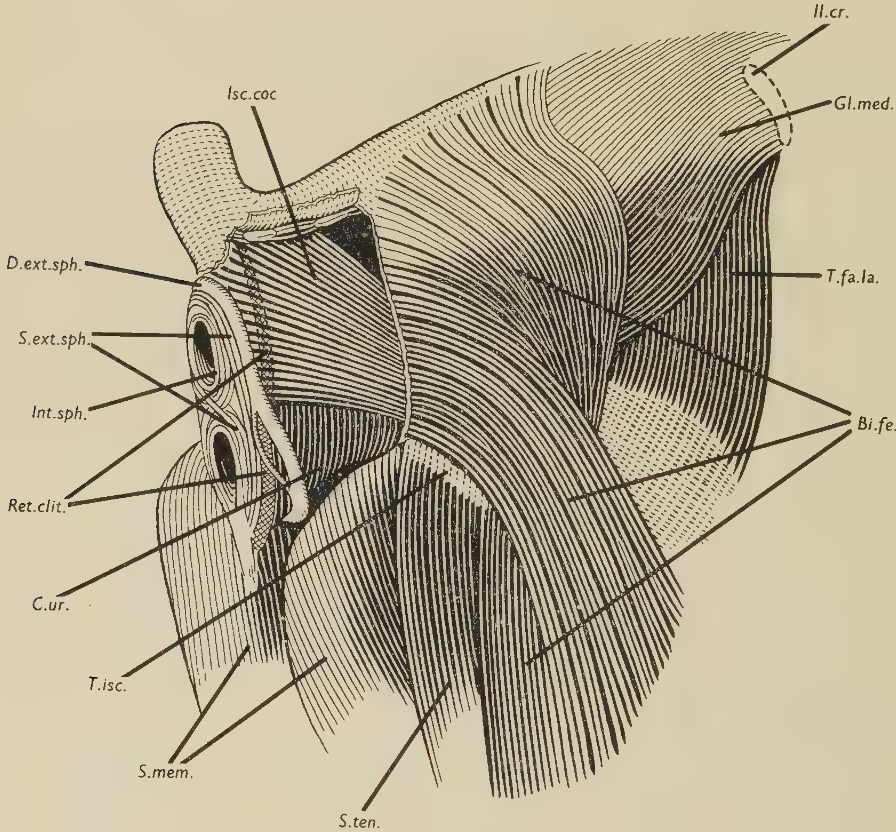
Morphological observations were made on the retractor clitoridis or retractor penis muscles of six cattle, four goats, eleven pigs and on small numbers of cats, rabbits, guinea-pigs, rats, mice, hedgehogs and phalangers (Australian 'opossums').

OBSERVATIONS

(1) *The retractor clitoridis muscle in the mature ewe*

(*a*) *Gross anatomy*

Since the proximal attachment of the retractor clitoridis is medial to the insertion of the ischiococcygeus muscle, this muscle, and the fat and fascia covering it, must, therefore, be removed before this part of the retractor clitoridis is seen (Text-fig. 1). It appears as a whitish band attached to the fascia under the tail, in the area of the



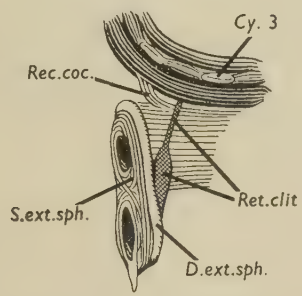
Text-fig. 1. Laterocaudal view of the external pelvic and perineal muscles of a mature ewe, with fascia and fat removed.

third–fourth (or occasionally between second–third) coccygeal vertebrae; here it is closely associated with the unpaired rectococcygeus muscle which is attached, at the midline, to the ventral tail surface slightly caudal to the origin of the retractor clitoridis (Text-fig. 2).

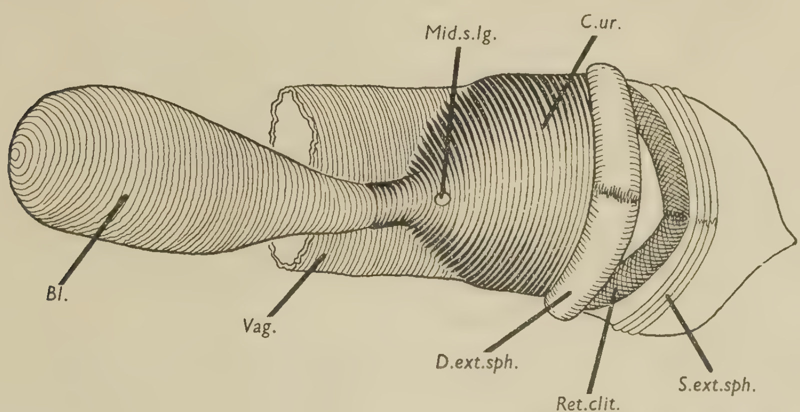
The retractor clitoridis muscle runs caudoventrally from its coccygeal attachment to the lateral wall of the caudal rectum, to which it adheres beneath ischiococcygeus. It then continues between the deep external sphincter muscle and the

rectal and vulval walls to terminate ventral to the vulva at the base of the clitoris where it meets its fellow from the opposite side (Text-fig. 3). The body of the muscle has a well-defined connective tissue sheath, and can be readily separated from surrounding structures. It is somewhat flattened in its rectococcygeal part, but the rectoclitoral part is characteristically cylindrical.

Although this was the general arrangement of the muscle in all ewes, there were, nevertheless, some minor variations. For example, in a foetus of 127 days, the rectococcygeal part was split into two at its proximal attachment. In one ewe, the rectoclitoral part divided as it passed ventrally from the wall of the vulva, and in another a small bundle of fibres left the main muscle at the junction of rectal and vulval walls, to pass dorsal to the anus and mingle with the fibres of the external sphincter.



Text-fig. 2. Laterocaudal view of the dissected perineal region of an immature ewe, showing the relation of the retractor clitoridis muscle to the rectococcygeus and perineal sphincter muscles.



Text-fig. 3. The relation of the retractor clitoridis muscle to the perineal and constrictor urethrae muscles at the ventral surface of the urogenital tract.

The clitoris itself is very insignificant. It is embedded in the tissue of the ventral wall of the vulva, the two crura being attached on either side of the ischial symphysis to a tough tendon which passes across the medial part of the ischial arch between the margins of the two bones (equivalent to the subpubic ligament in man).

Grossly, the muscle is innervated by a branch of the pudendal nerve, which arises

well within the pelvic cavity, about the level of the cranial margin of the ischiococcygeus muscle, and enters the retractor muscle at its attachment to the rectal wall. (This, however, does not preclude a possible dual innervation by the pelvic splanchnic nerves.)

The blood supply is from a branch of the internal pudendal artery, arising at a level with the caudal margin of the lesser sciatic notch.

(*b*) *Microscopical structure*

The muscle consists predominantly of bundles of smooth muscle fibres (Pl. 1, fig. 1), although small areas of striated fibres were scattered throughout the length of the serially sectioned whole muscle, and were also a feature of the clitoral terminations of muscles from several other animals (Pl. 1, fig. 4). The muscle fibre bundles are separated by aggregations chiefly of collagenous connective tissue, although many elastic fibres are also present (Pl. 1, fig. 2). There appears to be more connective tissue and a greater number of small blood vessels and nerves than is the case in the deep external sphincter (Pl. 1, fig. 3), a striated muscle of similar size in the same region (Text-figs. 1–3).

(2) *Development of the retractor clitoridis muscle in sheep*

(*a*) *Early prenatal*

The retractor clitoridis muscle is first discernible at 21 mm. as a diffuse thickening in the mesenchyme at the side of the rectum (Pl. 3, fig. 9) extending into the proximal part of the clitoris. At this stage, the rectum and urogenital sinus are completely separated, smooth muscle has formed in the wall of the rectum, but not in the urogenital sinus, the rudimentary skeletal muscles are quite distinct, but the external sphincter muscle complex is represented only by a slight thickening dorsal to the anus.

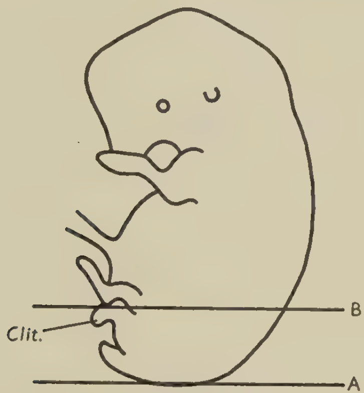
The retractor clitoridis becomes progressively more clearly defined; at 36 and 46 mm. it is the most distinct of all the perineal muscles, although the rectococcygeal part has not yet appeared.

By 46 mm. (Text-fig. 5 and Pl. 2, figs. 5–8) the *anlage* of the external sphincter has spread lateroventrally from the dorsum of the anus to embrace the lateral walls of the rectum and urogenital sinus, but does not yet continue round the anus. The rectococcygeus muscle is also incomplete, as it still has no coccygeal attachment. The ischiocavernosus muscle, on the other hand, is very well developed in these early stages, although it cannot be distinguished at 21 mm. it is completely formed by 26 mm. extending from the lateral ischial margin to the base of the clitoris.

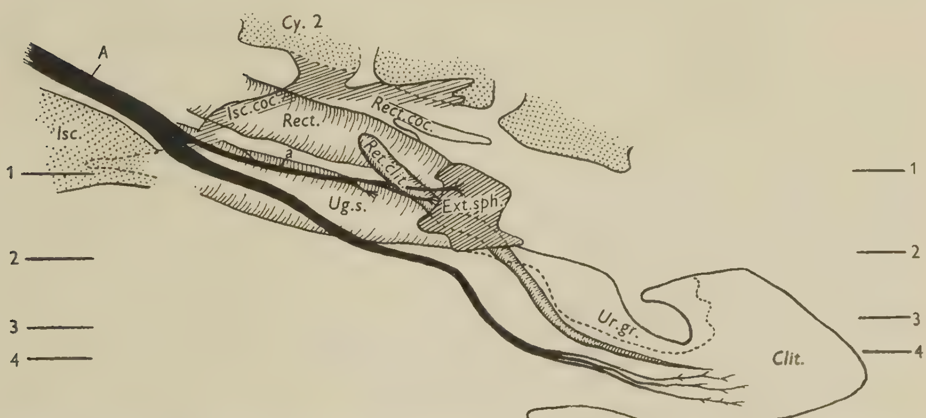
The chief innervation of the retractor clitoridis, as seen in the 36 mm and 46 mm. foetuses, is by a branch of the pudendal nerve, which arises at a level with the cranial margin of the ischiococcygeus muscle, then sends a twig to the skin near the tail (Pl. 2, fig. 5) before dividing to enter the retractor clitoridis near its origin at the rectal wall, and the external sphincter muscle (Text-fig. 5 and Pl. 3, fig. 10). The blood supply is by a small branch of the internal pudendal artery.

(b) Late prenatal and postnatal development

Nearer term (from 70 days onwards), although the clitoris is now relatively much smaller than in the early stages, the cylindrical rectoclitoral part of the retractor muscle is nevertheless strongly developed. The rectococcygeal part is now attached to the ventral surface of the tail but is yet very poorly developed. This distinction,



Text-fig. 4. Traced outline of a 36 mm. foetus. The region from which serial sections of all foetuses were examined is shown between lines A and B. ×1·4.



Text-fig. 5. Lateral graphical reconstruction of a 46 mm. foetus, showing the retractor clitoridis muscle descending from the rectal wall into the large clitoris, the innervation of the muscle by a branch of the pudendal nerve (black), and its relation to the external sphincter and the ischiococcygeus muscles (only cranial margin and ischial origin shown). Lines 1–4 indicate positions of sections illustrated in Pl. 2. ×18.

which is still present in young unbred animals (including the ram), is not evident however, in the mature ewe; in one case, in fact, in a ewe of 8 years which was at term, the rounded muscles from each side were nearly 1 cm. in diameter for their full extent from the coccygeal to the clitoral attachment.

Although the retractor clitoridis muscle remains a distinctive structure in the

mature ewe, in spite of the regression of the clitoris, the ischiocavernosus muscle diminishes as development proceeds. Usually, it is absent postnatally; if it exists it is only as a very insignificant muscle.

(3) *The effects of pregnancy in the ewe*

(*a*) *Weight changes*

The standard deviations show that there is considerable range in the variability of weight of the retractor clitoridis between ewes at the various gestational and involutionary stages, i.e. in some the muscle appears to undergo much greater change than in others. This conclusion is supported by comparison of the variance (Table 1*b*) within control subgroups (0·035) with that of all other groups combined (0·438).

Table 1. *Gestational changes in weight of retractor clitoridis muscle*

(*a*) Means and variability

Reproductive condition	Group	No. of ewes	Mean muscle weight g	S.D.	Mean carcass weight g	Date of slaughter
Control,	(1)	3	1·80	0·11	28,910	8. vii. 57–19. vii. 57
Non-pregnant, Dioestrous	(2)	3	2·50	0·24	33,720	23. ix. 57–24. ix. 57
Total	(1+2)	6	2·13	0·42	31,320	
Pregnant	5 weeks	6	2·23	0·40	24,250	3. v. 57–10. v. 57
	10 weeks	6	2·15	0·51	24,210	28. v. 57–13. vi. 57
	15 weeks	6	3·25	0·73	27,290	24. vi. 57–3. vii. 57
	20 weeks	6	3·71	0·87	27,990	24. vii. 57–6. viii. 57
Post-partum	within 12 hr.	6	4·09	0·66	27,510	7. viii. 57–22. ix. 57
	1 week	6	3·45	0·63	28,220	12. viii. 57–19. viii. 57
	4 weeks	6	3·30	0·72	23,940	31. viii. 57–9. ix. 57

(*b*) Analysis of variance

Source of variation		df	Ms
Between groups		7	3·522**
Within groups			
Control	(1) *v.* (2)	1	0·743**
	'error'...	4	0·035
Other groups		35	0·438
Total		40	0·405

** $P < 0{\cdot}01$.

The analysis of variance shows highly significant overall differences between the mean muscle weights at the several stages. The trend through gestation shown in Table 1*a* indicates that the increase in muscle weight first occurs between 10 and 15 weeks and reaches its maximum immediately after parturition. The mean weight then decreases during the month after lambing, although it is still higher than that of the controls.

The finding of a significant difference between the mean muscle weights of the two control subgroups (slaughtered at two different times approximately 2 months apart) implies a temporal change in the weight of the muscle, independent of reproductive condition. Caution is necessary, therefore, in ascribing the observed trends entirely to the stage of gestation. Although it seemed possible at first that these

differences in muscle weight between the two non-pregnant groups might be related to differences in size of the whole animal, analysis of covariance of retractor clitoridis weight with carcass weight yielded no evidence of significant association between them. The difference between means of the control subgroups remained significant when allowance was made for differences in carcass weight. A subjective assessment of the condition of the two lots of animals at time of slaughter had suggested that ewes in the second group were fatter than those in the first. Chemical determinations on these same muscles showed a significantly higher content of both fat and water in muscles from group 2. It thus seems that although the weight of the muscle is not related to body size, it may be affected by the nutritional condition as well as by the reproductive state. In New Zealand there is considerably more grass available for grazing animals in September than there is in July.

(*b*) *Chemical changes*

Chemical analysis shows that the increase in wet weight of the muscles between 10 and 15 weeks is associated with a significant increase in weight of non-connective tissue protein and solids-not-fat. There is, however, no significant increase in absolute weight of connective tissue (collagen + elastin).

The weight of water increases significantly between 10 and 15 weeks; although the mean values show a progressive increase to a maximum at parturition the trend is not significant statistically. The percentage of water increases between 15 and 20 weeks, and moisture on a fat-free basis is higher at 20 weeks than at other times.

(*c*) *Microscopical changes*

The most marked difference in the microscopical structure of muscles from non-pregnant ewes as compared with those 20 weeks pregnant (a week before estimated parturition date) is in the fibroblast cells of the connective tissue. In non-pregnant ewes, the nuclei of these cells are small and dense, with no stainable cytoplasm (Pl. 3, fig. 11), whereas in the pregnant ewes nuclei of the fibroblasts are large, clear and ovoid in shape, and the cytoplasm, which is strikingly evident, stains heavily with haematoxylin and is strongly basophilic with azur II and toluidine blue (Pl. 3, fig. 12). A second remarkable difference between the two groups is that in muscles from the pregnant ewes numerous eosinophil leucocytes are present among the collagenous fibres (Pl. 3, fig. 12), whereas none can be found in the non-pregnant ewes. As with the weights, there was some variation between animals at each stage in the intensity of these changes in fibroblasts and leucocytes.

There was no marked difference between the two groups of animals in the other connective tissue components, although the collagenous fibres appeared to be somewhat less densely arranged in pregnancy. No mitotic figures were found in the nuclei of fibroblasts or smooth muscle cells.

(4) *The retractor penis and clitoridis muscles in some other species*

(*a*) *Cattle*

The structure, arrangement and attachments of the retractor clitoridis muscle is the same as in the ewe; the two parts—rectococcygeal and rectoclitoral—are likewise continuous. The proximal attachment is to the fascia beneath the second and

third coccygeal vertebrae and the muscle, composed of smooth muscle fibres, passes between the rectal part of the ischiococcygeus muscle and the rectum to terminate at the base of the clitoris.

In the bull calf, the muscle is attached proximally to the ventral tail surface between the third and fourth coccygeal vertebrae. The rectococcygeal part is continuous with, although somewhat smaller in diameter than, the rectopenile part. The muscles from each side join at a level with the ischial symphysis, to continue between the legs along the ventral surface of the penis (i.e. ventral with respect to its position fixed within the abdominal wall).

(*b*) *Goat*

The arrangement is again similar to that in sheep and cattle, the only variation being in the exact position of the coccygeal attachment, which in the female (both foetal and mature) is to the fascia of the ventral tail surface, between the second and third coccygeal vertebrae, while in the male it is between the third and fourth coccygeal vertebrae. In the female foetuses and in the male the rectococcygeal part of the muscle is less well developed than the rectoclitoral or rectopenile parts, the junction being discernible on the lateral rectal wall, as in the immature ewe. In the mature female both parts are well developed. Smooth muscle fibres are present.

(*c*) *Pig*

The retractor clitoridis and penis muscles are rather less strongly developed than in the sheep, cattle and goats. The position of the proximal attachment differs in the male and the female. In the male, the retractor penis from each side attaches to the fascia of the ventral sacral surface, immediately cranial to the joint between the third and fourth sacral vertebrae, and since the ischiococcygeus muscle has no rectal attachment it passes caudally alongside the rectum until it reaches the level of the external anal sphincter, then passes ventrally to join the penis at the margin of the ischial symphysis. In the female the rectococcygeal part is absent; the rectoclitoral part passes from the lateroventral rectal wall to the base of the clitoris, which is a very insignificant structure.

In both sexes smooth muscle fibres are present and the muscle is innervated by the pudendal nerve.

(*d*) *Cat*

The retractor clitoridis is a white rounded band of smooth muscle attaching to the ventral surface of the tail at the level of the first coccygeal vertebra. It adheres by its fascia to the side of the rectum and then descends to the ventral surface of the vulva, at the base of the clitoris.

(*e*) *Rabbit*

In the rabbit there appears to be no muscle comparable with the retractor penis or clitoridis of the other species. There is, however, a band of striated fibres passing from the rectal wall to the base of the penis, and in two animals a second band of striated muscle was observed, covering the other and extending from the fascia on the ventral surface of the fifth coccygeal vertebra to the base of the penis.

(f) Guina-pig

In both male and female a pale band of smooth muscle passes from the latero-ventral rectal wall to the penis or clitoris. There is no rectococcygeal continuation of the muscle.

(g) Rat

The perineal muscles in the rat are not clearly distinguishable. A pale band of striated muscle could be seen passing from the ventral surface of the third coccygeal vertebra to the flexure of the penis.

(h) Mouse

Here too, the perineal muscles are seen only with difficulty, even with a dissecting microscope. In both males, two bands of smooth muscle were observed, extending from the ventrolateral rectal wall to the ventral surface of the penis. Traction with forceps on the cut rectal endings of these muscles was effective in retracting the extended penis.

(i) Hedgehog

A retractor penis or clitoridis is clearly present, but the rectococcygeal part is absent. The muscle arises proximally from the lateroventral rectal wall at a level with the margin of the transverse process of the third coccygeal vertebra. In the male the muscles from each side insert into the last quarter of the penis, and in the female they at least reach the base of the clitoris.

(j) Phalanger (Australian opossum)

In these animals the retractor penis, composed of smooth muscle, is very well developed. It is attached to the ventral surface of the first coccygeal vertebra. In the phalanger the sacrum has only two vertebrae, and the first coccygeal vertebra is at a level with the cranial margin of the acetabulum. The origin of the retractor penis is thus much further craniad than in the other species described; therefore, it passes horizontally along the rectal wall for some distance, before being deflected ventrally to join the penis.

DISCUSSION

Although the form and position of the retractor clitoridis muscle in the ewe is similar to striated muscles such as the closely associated perineal sphincter, it has been shown to have several characteristic differences in microscopical structure. Not only are the fibre bundles largely composed of smooth muscle, but also they are interspersed with more connective tissue and there are numerous small nerve fibres and blood vessels within its body in comparison with the normal distribution within striated muscle, in the same region at least.

The findings concerning this muscle suggest that in the female it may possess a function other than that indicated by its name, for, in spite of the regression, with maturity, of the clitoris and the ischiocavernosus muscle, the retractor clitoridis remains characteristically well formed with an abundant nerve and blood supply.

In some species the retractor penis or clitoridis muscle has been shown to be under control of the autonomic nervous system and hence to possess the involuntary activity typical of smooth muscle elsewhere. Thus, whatever additional function the retractor clitoridis may have, it is likely to be also of an involuntary nature. The relation of the muscle to the vagina suggests that it could constrict the orifice of this organ during copulation, or at least enhance the action of the sphincter-like structure, also of smooth muscle, which is present within the vaginal walls immediately cranial to the urethral orifice. This hypothesis, however, requires experimental investigation.

The striated muscle fibres which are found among the smooth muscle bundles are of some interest, as it is unusual to find a mixture of the two types of muscle fibre in such close association. Langley & Anderson (1895) described striated muscle within the retractor penis of the dog and cat, and considered that these fibres may be derived from the external anal sphincter and the bulbocavernosus muscles. There is no evidence in the present investigations for such a developmental possibility. In the rat and rabbit, in the position occupied by the retractor penis in other species a bilateral structure is found which is composed, predominantly at least, of striated muscle, but as investigations on the innervation and function of these muscles were not carried out, no comment can be made concerning their homology. In all the other species investigated here the retractor penis, or clitoridis, was composed of smooth muscle.

Study of the development of the retractor clitoridis muscle has provided some new information concerning it. In the ewe it is primarily associated with the clitoris, for it is strongly developed in the early embryo when this organ is relatively very large. The principal origin is from the lateral wall of the terminal rectum, the attachment extending to the coccygeal vertebrae only later in development, somewhere between the 46th and 70th day. The distinction between the two parts is also quite obvious in young unbred ewes, in which the rectoclitoral portion is still noticeably larger than the rectococcygeal part. The facts that the muscle develops in two such distinct parts in the sheep and that in several other species there is no coccygeal attachment even in the mature animal, suggests that the rectococcygeal part is both phylogenetically and ontogenetically a secondary development. Observations on the pig indicate that in some species there may also be a sex difference in the extent of development of the muscle.

The various pregnancy changes in the ewe are not confined to the retractor clitoridis muscle. A gestational weight increase has been recorded also for the ischiococcygeus and deep perineal sphincter muscles; in the former this was shown to be associated, in part at least, with the increase in muscle fibre diameter (Bassett, 1956 and unpublished work).

Newbold's finding of an increase in non-connective tissue protein (largely smooth muscle) of the retractor clitoridis muscle is in line with the report of Needham & Cawkwell (1956) concerning the sarcoplasm fraction of the pregnant rat's uterus. In pregnancy there is hyperplasia and hypertrophy of uterine smooth muscle fibres. Experimental evidence shows that both hormones and physical distension stimulus can cause hyperplasia, though the relative effects of these two factors on hypertrophy seem less well known (Reynolds, 1949, 1951).

Although no references can be found dealing with the effects of pregnancy on whole muscle weight, castration and androgen treatment in some rodents have been shown respectively to decrease and increase the size of the levator ani (= ischio-coccygeus) and other perineal muscles, including the retractor penis (Wainman & Shipounoff, 1941, who also related these effects to change in fibre width; Eisenberg & Gordon, 1950; Kochakian *et al.* 1956; Kochakian & Tillotson, 1957; Kochakian, Tillotson & Austin, 1957).

It can be inferred that increase in weight of smooth and striated perineal muscles during pregnancy, whether by hypertrophy or hyperplasia, is effected largely by endogenous hormones. In the ewe, this hypothesis is strengthened by the fact that whole weight and non-connective tissue protein changes in the retractor clitoridis, and weight increase in other perineal muscles, become apparent at about 15 weeks of gestation, which is the stage when oestrogenic and androgenic hormones can first be detected in the urine (Bassett, Sewell & White, 1955).

The relative decrease in connective tissue weight of the retractor clitoridis muscle during pregnancy is in agreement with findings of other investigators of gestational connective tissue changes in the reproductive tract (Needham & Cawkwell, 1956; Harkness, 1955–56, 1957). Harkness has suggested qualitative differences either in the ground substance or in the collagenous fibres themselves to account for 'relaxation' of pelvic connective tissue structures during pregnancy. The microscopical changes in the connective tissue in the retractor clitoridis muscle and in other pelvic tissues of the ewe (Bassett, 1956, 1958) support this hypothesis. The marked basophilia of the fibroblast cells denotes intense activity—probably that of breaking down mature collagenous fibres and forming new ones and contributing to the increase in matrix (Bassett, 1959). Storey (1957) has described similar modifications in the symphyseal ligament of the pregnant mouse.

The accumulation of eosinophil cells which was seen in the retractor clitoridis of the pregnant ewe has been found also in all the pelvic tissues described above, except the sacroiliac joint (Bassett, 1956); it also occurs in the uterine wall of ewes at term (unpublished work). No other references could be found concerning this phenomenon, although leucocytes other than eosinophils have been studied in several species during pregnancy; for instance, Hofbauer (1926) mentions the appearance of monocytes, clasmatocytes and 'pseudo-eosinophils' in the broad (uterine) ligament, and Fluhmann (1928) found an increase in macrophages in the uterus. It is known, however, that there is a decrease in number of eosinophils in the circulating blood at the end of pregnancy (Paterson, 1957; Marcus, Cibley, Brandt, Millman & Barlas, 1958; Moore, 1958), and also that hormone administration (chiefly adrenal hormones) has an effect on eosinophils and other leucocytes in the blood (see Dougherty, 1952; Dougherty & Dougherty, 1953; and Gordon, 1954, for reviews). So it is not improbable that the concentration of eosinophils in certain tissues in pregnancy is also affected by endogenous hormones. It seems likely that this gestational increase in eosinophils in the tissues takes place by removal of these cells from the blood, but as the specific function of eosinophils is uncertain (Code & Mitchell, 1957; Speirs, 1958), the purpose of such a transfer is a matter for conjecture.

The observations recorded suggest that the retractor clitoridis muscle may be of importance to the animal in two different ways. In view of its homology with the

male muscle, its strong development and type of innervation, and its position with relation to the vagina, it could have an involuntary action, probably of quite short duration, with respect to the copulation process. Adequate functioning in such a manner may be necessary for conception. Since it undergoes pregnancy changes similar to those in other perineal structures, it must share with them also a more passive role in enlargement of the pelvic outlet at parturition.

SUMMARY

The mammalian retractor penis muscle and its female homologue, the retractor clitoridis, both composed largely of smooth muscle, are found only in species having the penis fixed in the abdominal wall.

A detailed study has been made of the retractor clitoridis muscle in a large number of ewes (*Ovis*), including its morphogenesis, morphology, microscopical structure and alterations in pregnancy. The morphology of the retractor penis and clitoridis muscles has been observed also in various other species, including cattle (*Bos*), goat (*Capra*), pig (*Sus*), cat (*Felis*), rabbit (*Oryctolagus*), guinea-pig (*Cavia*), rat (*Rattus*), mouse (*Mus*), hedgehog (*Erinaceus*), and phalanger (Australian opossum: *Trichosurus*).

The muscle takes origin primarily from the lateral rectal walls, the coccygeal attachment present in many species, including the sheep, being both phylogenetically and ontogenetically a secondary one; it is absent in the sow (though not the boar), hedgehog, guinea-pig and possibly the mouse.

In the ewe, the muscle contains largely smooth muscle but also some striated muscle fibres, much connective tissue including collagenous and elastic fibres, and many small nerves and blood vessels. The gestational effects include an increase in whole muscle weight, great activity of fibroblast cells, and an accumulation of eosinophil leucocytes.

Since the muscle is so well developed in spite of the small size of the clitoris, it may have some additional involuntary (? sphincteric) function other than that suggested by its name.

The observations recorded in this paper, and which are part only of a much larger study, were made in two different laboratories and written up in yet a third. Detailed acknowledgement of all the people who have rendered assistance would thus be out of proportion to the length of this text.

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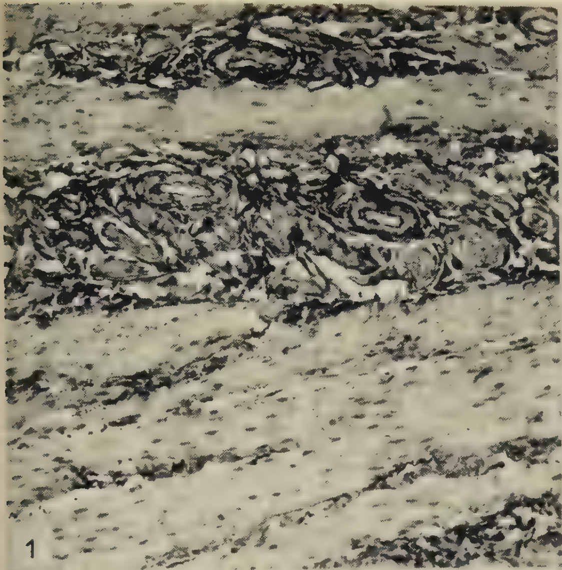

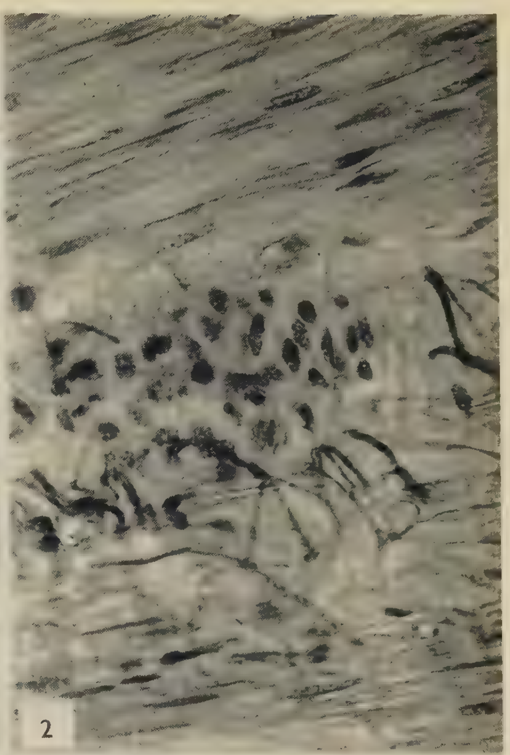

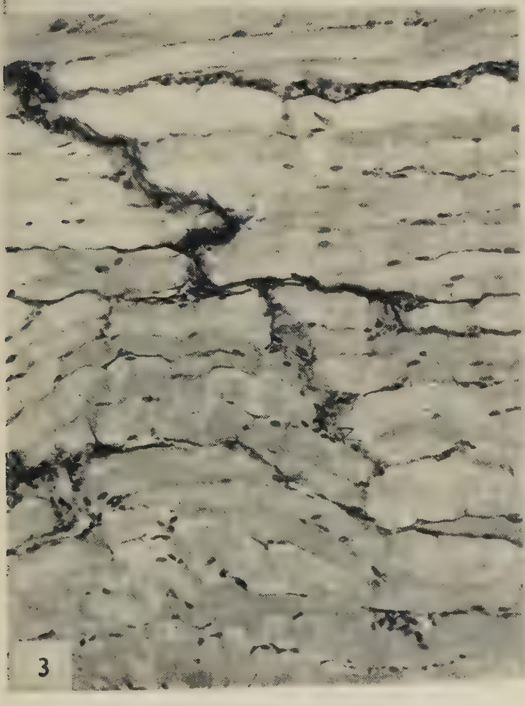

BASSETT—RETRACTOR PENIS AND RETRACTOR CLITORIDIS MUSCLES

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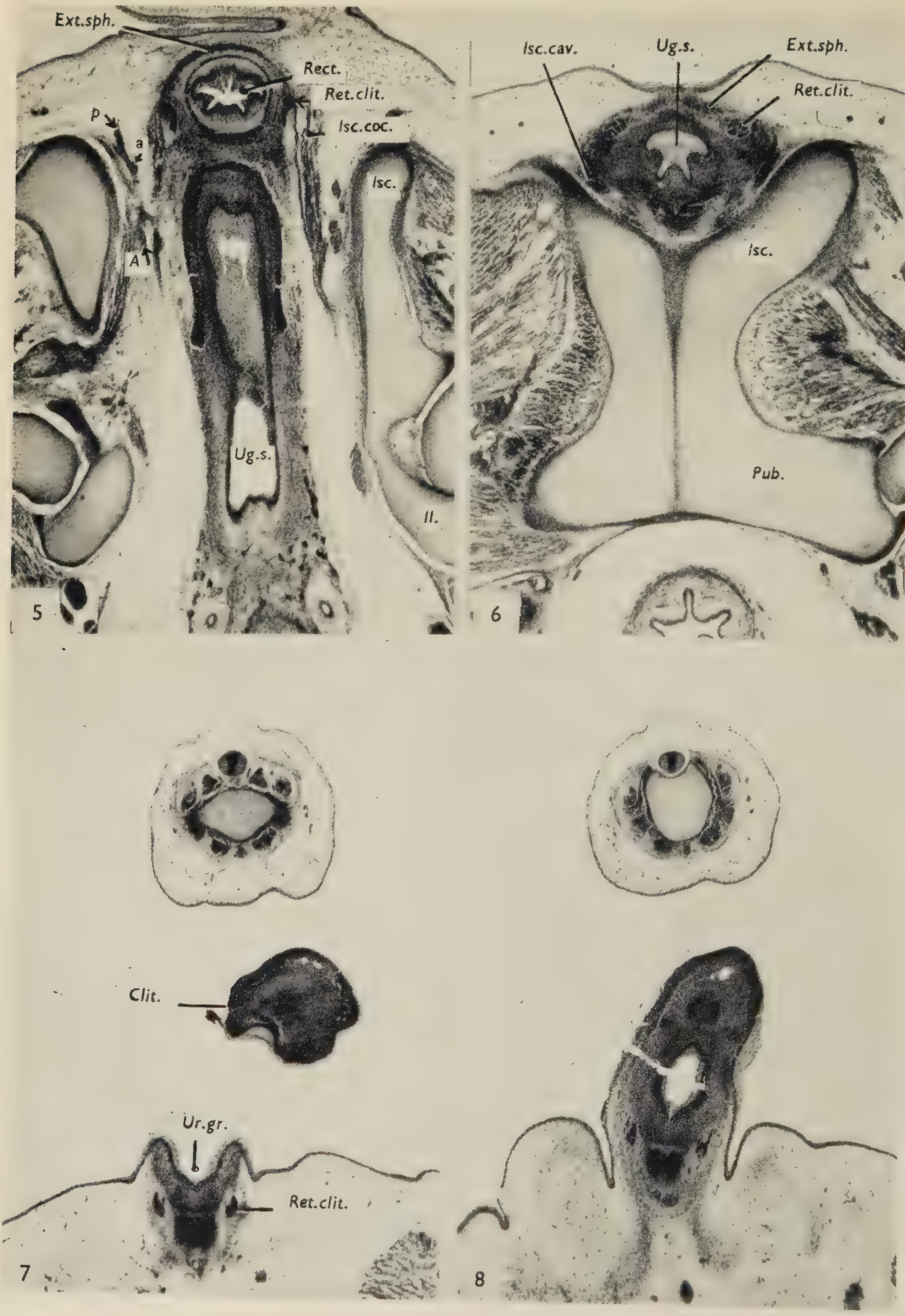

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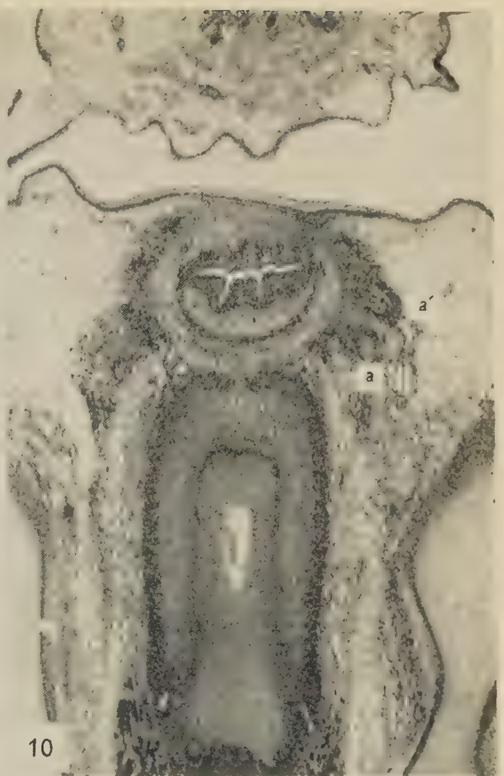

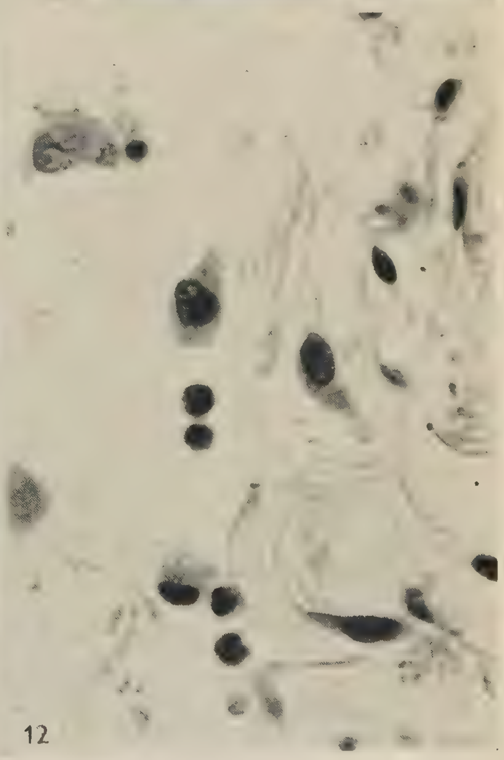

BASSETT—RETRACTOR PENIS AND RETRACTOR CLITORIDIS MUSCLES

KEY TO ABBREVIATIONS USED IN FIGURES

A	Pudendal nerve
a	Branch of pudendal nerve
Bi.fe.	Biceps femoris
Bl.	Bladder
Clit.	Clitoris
C.ur.	Constrictor urethrae
Cy. 2, *Cy.* 3	Coccygeal vertebrae
(*D*)*ext.sph.*	(Deep) external sphincter
Gl.med.	Gluteus medius
Il.	Ilium
I.cr.	Iliac crest
Int.sph.	Internal sphincter
Isc.	Ischium
Isc.cav.	Ischiocavernosus
Isc.coc.	Ischiococcygeus
Mid.s.lg.	Midsymphyseal ligament
Pub.	Pubis
Rect.	Rectum
Ret.clit.	Retractor clitoridis
Rec.coc.	Rectococcygeus
S.ext.sph.	Superficial external sphincter
S.mem.	Semimembranosus
S.ten.	Semitendinosus
T.fa.la.	Tensor fasciae latae
T.isc.	Tuber ischii
Ug.s.	Urogenital sinus
Ur.gr.	Urethral groove
Vag.	Vagina.

EXPLANATION OF PLATES

Plate 1

All sections are longitudinal and stained with Weigert's haematoxylin, van Gieson and orcein.

Fig. 1. The retractor clitoridis muscle. Note bundles of smooth muscle fibres, interspersed with connective tissue containing many small blood vessels and nerves. ×150.

Fig. 2. High-power view of section shown in Fig. 1, showing smooth muscle fibres at top and bottom, collagenous and elastic fibres at centre. ×405.

Fig. 3. Deep external perineal sphincter muscle; compare general structure with Fig. 1. ×150.

Fig. 4. Striated muscle fibres among smooth muscle bundles in retractor clitoridis muscle. ×90.

Plate 2

Frontal sections from foetus reconstructed in Text-fig. 6. All stained with haematoxylin and eosin. ×22.

Fig. 5. Line 1 in Text-fig. 6. Retractor clitoridis is attached to the rectum medial to the rectal insertion of ischiococcygeus (right). The external sphincter is on the dorsum of the rectum. Branch (*a*) of the pudendal nerve (*A*) is sending a twig (*p*) to the skin near the tail (left).

Fig. 6. Line 2. Cross-sections of the retractor clitoridis *anlagen* are clearly defined at the lateral sinus walls. Ischiocavernosus *anlagen* are at the caudal ischial margins.

Fig. 7. Line 3. Shows the cranial portion of the urethral groove and the tip of the clitoris. Retractor clitoridis *anlagen* at sides of groove.

Fig. 8. Line 4. Shows the retractor clitoridis in undifferentiated mesenchyme of clitoris.

Plate 3

Fig. 9. Frontal section through pelvic region of 21 mm. foetus, at same level approximately as line 1 in Text-fig. 6. Retractor clitoridis *anlagen* discernible at lateral rectal walls. ×45.

Fig. 10. Section from 36 mm. foetus, at a level between lines 1 and 2 in Text-fig. 6. Retractor clitoridis and external sphincter innervated by two twigs (*a*, *a'*) of branch of pudendal nerve. ×45.

Fig. 11. Longitudinal section through connective tissue of retractor clitoridis muscle of non-pregnant ewe. Note fibroblast cells with small dense nuclei and no stainable cytoplasm. Haematoxylin-azur II-eosin. ×675.

Fig. 12. Section from pregnant ewe (20 weeks) comparable with Fig. 11, showing enlarged nuclei and marked cytoplasmic basophilia of fibroblast cells. Several eosinophil cells are present also. ×675.

THE THALAMIC PROJECTION UPON THE TELENCEPHALON IN THE PIGEON (*COLUMBA LIVIA*)

By T. P. S. POWELL and W. M. COWAN

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INTRODUCTION

The exceptional development of the striatum in the avian telencephalon is unique amongst vertebrates and for this reason is of considerable interest to the comparative neuroanatomist. Associated with this unparalleled striatal development is the extreme reduction in size of the 'cortical' areas of the cerebral hemisphere. Nevertheless, the thalamus is well differentiated and is composed of a number of well-developed nuclei. The striking difference between the thalamic development and that of the cortex is surprising in view of the intimate thalamocortical relationship found in the mammal and suggests a threefold problem. First, can the well-differentiated avian thalamus be predominantly related to the poorly developed cortex (i.e. is the thalamic projection similar in principle—if not homologous—to that of the main thalamic nuclei in mammals)? A second possibility is that all or most of the thalamic nuclei of the bird project upon the striatum and form a system similar to that which has recently been described in the mammal. The third possibility to be considered is that the projection of the avian thalamus is an evolutionary specialization without an equivalent in mammals. It is difficult on *a priori* grounds to know which of these possibilities is the more likely to be correct, especially as so little is known of the functional significance of either the striatum or the thalamus in birds. However, it is obvious that the unique development of the avian striatum must be related to its distinctive mode of life, and a clearer knowledge of the connexions and organization of the striatum might help in the elucidation of its functional significance. In addition to this, any light thrown upon the morphology of the striatum will be of value in reconstructing the mode of evolutionary development of the forebrain.

There is a considerable literature dealing with the normal morphology of the forebrain in birds (Kappers, Huber & Crosby, 1936), but little work has been done using the conventional experimental anatomical techniques. The necessity for an experimental investigation of this kind need hardly be emphasized as it is well known that connexions can only be established with certainty using such methods. In this study we have attempted to define the projection of the thalamic nuclei upon the telencephalon by the method of retrograde cell degeneration which has been used so successfully in the mammal. The finding of retrograde cell degeneration in a given structure can be accepted as unequivocal evidence for a projection to the site of the lesion, but a negative result, of course, must be interpreted with caution.

In the present paper the results are presented in two sections: in the first the total thalamic projection upon the telencephalon will be described for, as Rose & Woolsey (1943) have pointed out, there is a distinct advantage in determining the total

thalamic projection by studying the extent of the retrograde cell degeneration in the thalamus after complete removal of the telencephalon before attempting to define the precise projection of individual thalamic nuclei. In the second section the projection of some of the individual nuclei will be presented based upon an analysis of the retrograde degeneration following a variety of smaller telencephalic lesions. A preliminary account of these results was given some time ago (Powell & Cowan, 1957).

MATERIAL AND METHODS

Altogether seventy adult pigeons (*Columba livia*) were operated upon, and of these the brains of fifty-two were used. For determining the total thalamic projection four brains have been useful and the remainder for the localization studies presented in the second section. The animals were anaesthetized with open ether and the skull exposed through a midline skin incision. Craniotomies varying in size and position were made and the lesions produced either with a needle or by suction with a fine aspirator. The animals were allowed to survive for periods ranging between 1 and $2\frac{1}{2}$ months. They were killed by an overdose of ether and the brains fixed by immersion in 70 % alcohol and 2 % acetic acid. After embedding in paraffin wax the brains were cut at 25 μ in either the coronal or horizontal plane; a one-in-five series of sections was mounted and stained with thionine.

One of the unexpected features of this study was the ease with which lesions could be placed in the brain of the pigeon, and the remarkable ability to survive such extensive lesions as virtually complete removal of the telencephalon on one side. No detailed behavioural studies were made upon these animals, but we have been impressed by the absence of any overt functional deficit even after large lesions.

TERMINOLOGY

The terminology of the avian telencephalon and diencephalon has been clarified by Huber & Crosby (1929) and Kappers *et al.* (1936). These authors did not give an account specifically of the pigeon brain, but we have found that their detailed descriptions of other species, particularly the sparrow and dove, is so similar to the pigeon that a separate description of the normal morphology of the pigeon need not be given. For the same reasons we have followed the terminology of these authors with only minor modifications. For example, in our descriptions of the lesions in the telencephalon we have not differentiated between the various subdivisions of the hyperstriatum which they have described, not because they cannot be recognized in our material, but because we have found no evidence for differential connexions between the thalamus and these subdivisions. Similarly, although we have been able to differentiate the so-called ektostriatum from the adjacent neostriatum and palaeostriatum in normal material, it has not always been possible to recognize it clearly in the operated hemispheres, partly because of the resulting distortion, and partly because of distinct shrinkage of the constituent cells and accompanying gliosis. For these reasons we have normally included it with the neostriatum in the descriptions of the lesions.

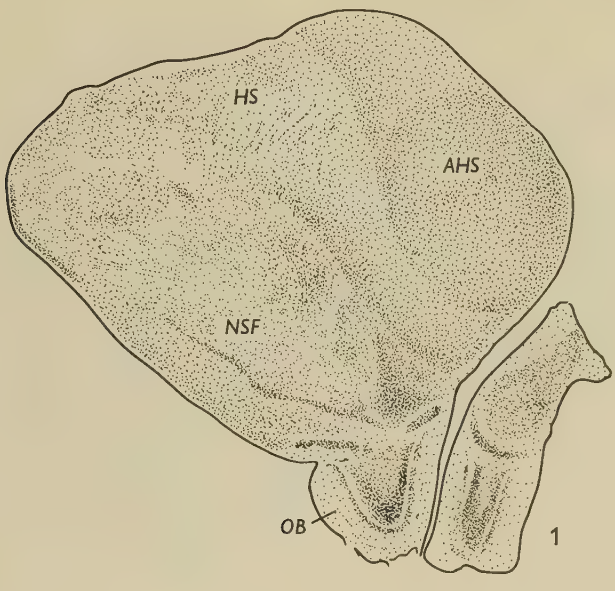
RESULTS

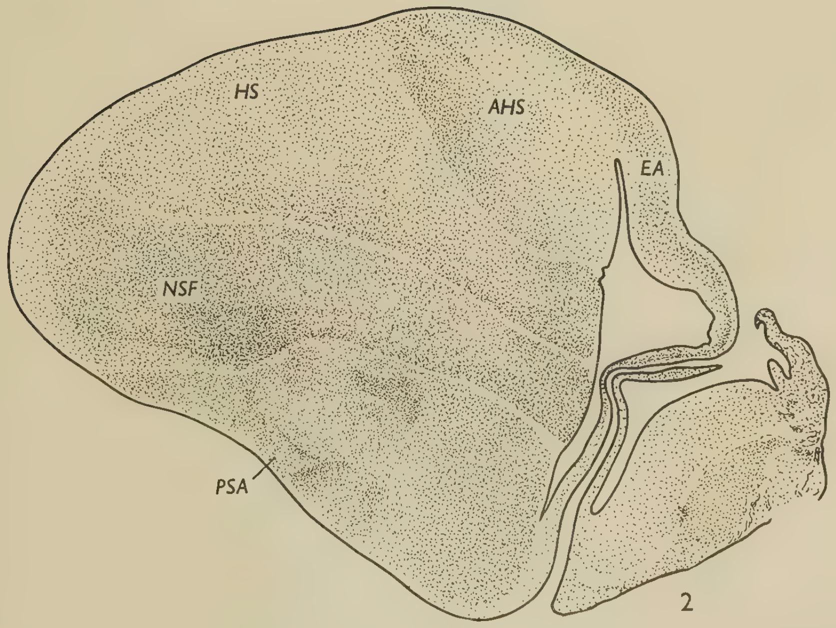
(I) *The total thalamic projection upon the telencephalon*

Before describing the results of the individual experiments some comments on retrograde cell degeneration in the avian thalamus may be apposite as this has not, to our knowledge, been previously described. Such cellular degeneration appears to be just as well defined and clear-cut as that found in the mammal, and in some of the affected nuclei has resulted in severe cell loss and gliosis. In other nuclei, however, such as the entopeduncular nucleus, the degeneration takes the form of a marked cell shrinkage and compacting of the cells rather than a cell loss and in this respect resembles the degeneration found in the thalamic reticular nucleus in the mammal (Rose, 1952).

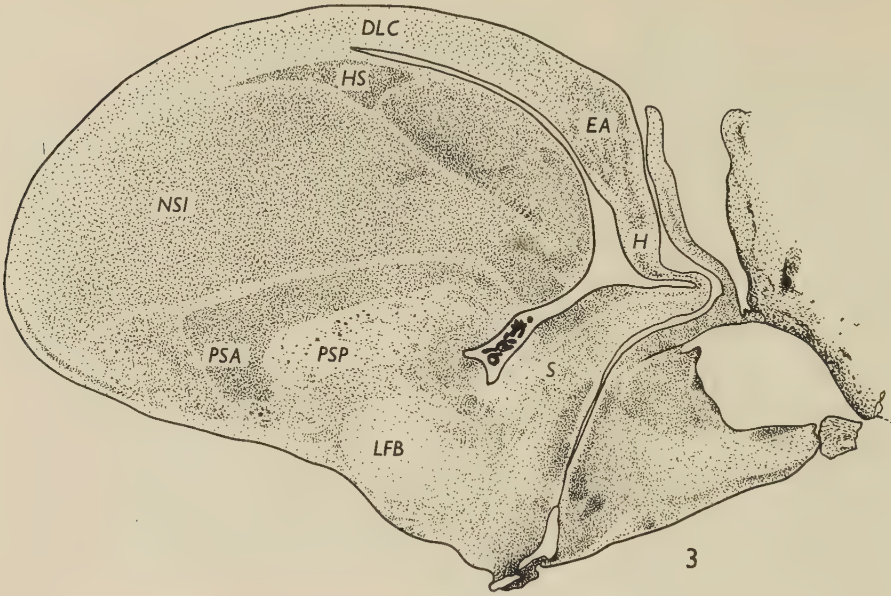
Experiment OP 20 is typical of an unilateral telencephalic ablation; in this experiment almost the entire telencephalon of the right side was removed by suction, and the animal was allowed to survive for 44 days. Examination of the serial sections shows that anteriorly only a small fragment of the ventromedial corner of the hemisphere adjacent to the inferior angle of the ventricle remains, and posteriorly there is, in addition, a small remnant of cortex (Text-fig. 1; Pl. 1, fig. 1). The parts remaining include the posterior part of the olfactory bulb, together with the most medial part of the prepyriform cortex, anterior olfactory nucleus and the ventralmost part of the hyperstriatum. At a slightly more posterior level this ventromedial fragment consists of the degenerated nucleus of the diagonal band and the most medial portion of the palaeostriatum. Just caudal to the anterior commissure (Text-fig. 3) the only parts of the telencephalon which are preserved are the posterior portion of the septum, and the hippocampal and entorhinal areas of the cortex on the medial surface of the hemisphere. The septo-mesencephalic tract has been severely damaged, and it is markedly shrunken and gliosed. Ventral to the anterior commissure the medial preoptic area and the medial part of the lateral preoptic area are intact. More caudally (Text-figs. 4, 5), above the diencephalon, the amount of cortex remaining progressively increases until it forms a complete ring at the posterior pole of the hemisphere. With the possible exception of slight marginal involvement of the dorsolateral edge of the thalamus the diencephalon is undamaged (Text-fig. 6). In summary, this experiment can be considered as a virtually complete telencephalic ablation.

From an analysis of the thalamic nuclei in this experiment it is clear that they fall into three distinct groups on the basis of their cellular reaction. In the nuclei of the first group the cells show profound retrograde degeneration; marked cell loss has occurred, and no normal cells remain. This group comprises the two most conspicuous elements of the avian thalamus, namely, the nucleus rotundus and the nucleus ovoidalis together with the nucleus dorsolateralis anterior (Text-figs. 6, 7; Pl. 1, fig. 2). The degeneration is most severe in the nucleus rotundus; only a small number of neurons persist, and these all appear considerably paler than normal, and are swollen and ill-defined (Pl. 2, figs. 3, 4). The severe cell loss and gliosis together serve to differentiate sharply the degenerated nucleus from the neighbouring cell masses. In the adjoining nucleus ovoidalis a somewhat larger proportion of cells remain, but again all of these cells are abnormal, being enlarged in size





Text-figs. 1, 2. Drawings to show the principal subdivisions of the telencephalon on the left and the extent of the lesion at anterior levels in experiment OP 20 (right-hand side). These and subsequent drawings of this experiment have been traced from transverse sections at intervals of 0·75 mm. using a projection apparatus. On the operated side only the ventromedial corner of the hemisphere remains.



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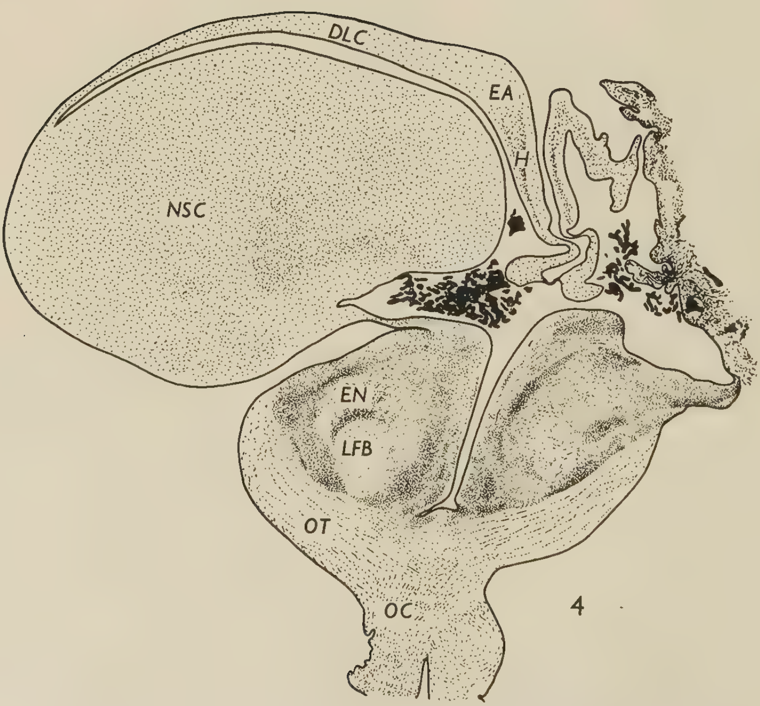

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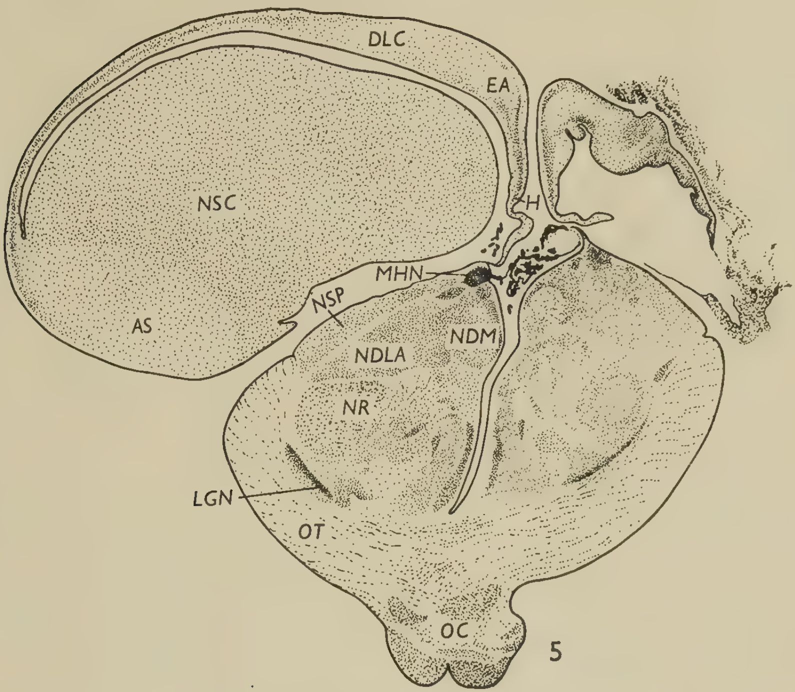
Text-figs. 3, 4. Drawings of the hemispheres of experiment OP 20 at the level of the middle of the septum and the anterior end of the thalamus, respectively.



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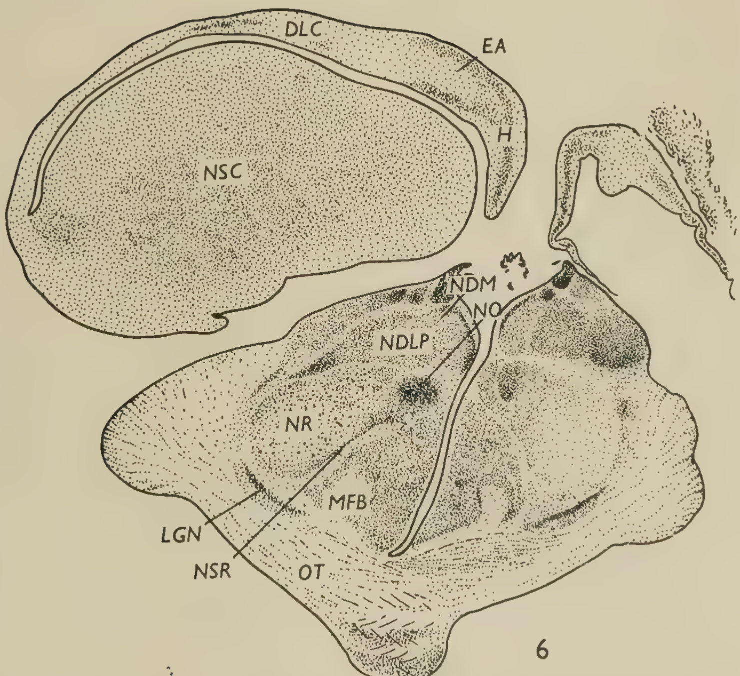

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Text-figs. 5, 6. Drawings at more caudal levels of the hemispheres of experiment OP 20 which show the extent of the lesion and the distribution of the relevant thalamic nuclei.

and paler staining; the accompanying gliosis is more marked in this nucleus (Pl. 3, figs. 6, 7).

Before describing the changes in the dorsolateral nucleus it is necessary to comment upon the morphology of this nuclear mass in the pigeon. The general form of the nucleus corresponds to the description given by Huber & Crosby (1929) for the dove. There is no sharp demarcation in coronal sections between what has been defined as the nucleus dorsolateralis anterior and the nucleus dorsolateralis posterior in other species. A gradient of cell size exists between the ventral and dorsal margins, however, on the basis of which it is possible to differentiate a larger-celled dorsal area and a ventral area containing smaller, darker-stained cells. In sections of the thalamus which are cut in the horizontal plane the differentiation is clearer, the small-celled area being almost circular in outline and situated anteromedially with the larger cells occupying a triangular area posterolaterally with its apex directed forwards. On the operated side of this experiment only the ventral area shows any degeneration; here marked cell loss has occurred, and the surviving cells are paler and shrunken; these cellular changes are accompanied by severe gliosis. This experiment therefore provides a valid criterion for subdividing the dorsolateral nucleus into distinct parts. We shall call the degenerated nucleus the nucleus dorsolateralis anterior and the nucleus which remains unaffected the nucleus dorsolateralis posterior, following the terminology of Huber & Crosby (1929). The preservation of the nucleus dorsolateralis posterior in this experiment might be interpreted as showing that this nucleus does not project upon the telencephalon. Evidence will be presented later to show that this is not so; cellular degeneration equally severe to that seen in the nucleus dorsolateralis anterior is found in this nucleus in other experiments.

In the second larger group of nuclei less marked, but unequivocal, changes are found. The changes vary from slight pallor of the constituent cells in some nuclei to pronounced cell shrinkage together with partial cell loss in other nuclei. The detailed changes will be described for the respective nuclei as these appear in an anteroposterior series. The cells of the two parts of the entopeduncular nucleus react differently but in neither does any cell loss occur; the large, deeply staining cells of the dorsal part appear much paler and are considerably reduced in size with less obvious processes. The smaller cells of the ventral part of the nucleus are also much paler on the operated side; they are unchanged in size, but they no longer have their characteristic spindle shape and appear distinctly globular. As a result of the degeneration of the fibres of the lateral forebrain bundle, the cells are closely compacted together and are surrounded by an intense gliosis. In the nucleus dorsomedialis anterior and the nucleus superficialis parvocellularis the cells are considerably paler than normal; it is also possible that the cells are slightly shrunken. A moderate degree of gliosis is present, but there is no suggestion of any cell loss in either of these nuclei. At low magnifications it would appear that the nucleus subrotundus has undergone complete cell loss, but at higher magnifications it is clear that this appearance is due to very marked cell shrinkage and pallor.

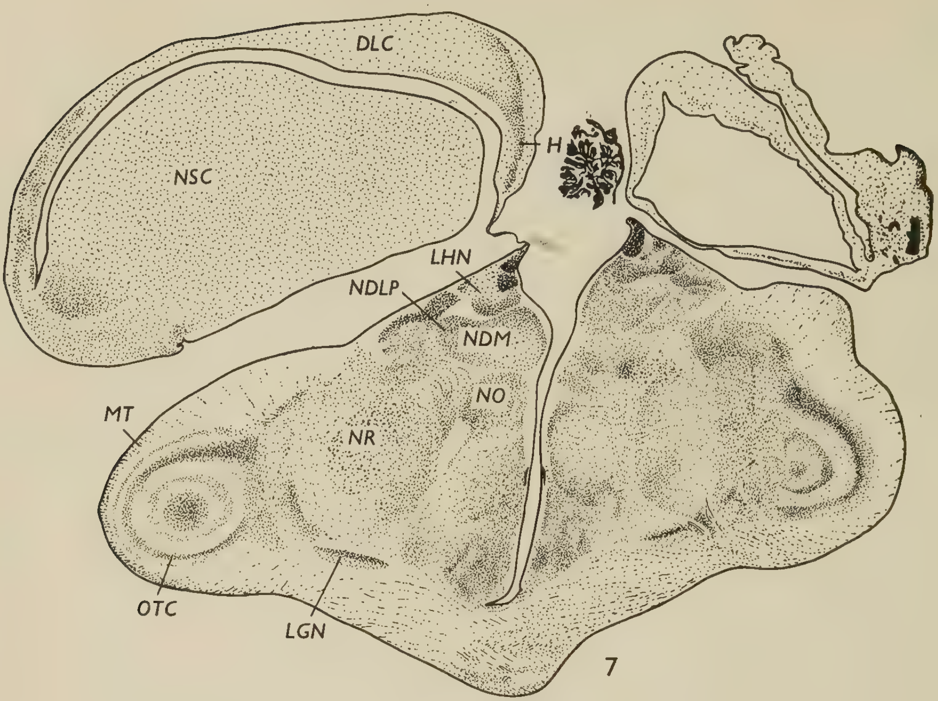
In the nuclear mass immediately caudal to the nucleus rotundus there are again two distinct cell types: in the large-celled lateral part of the nucleus (probably

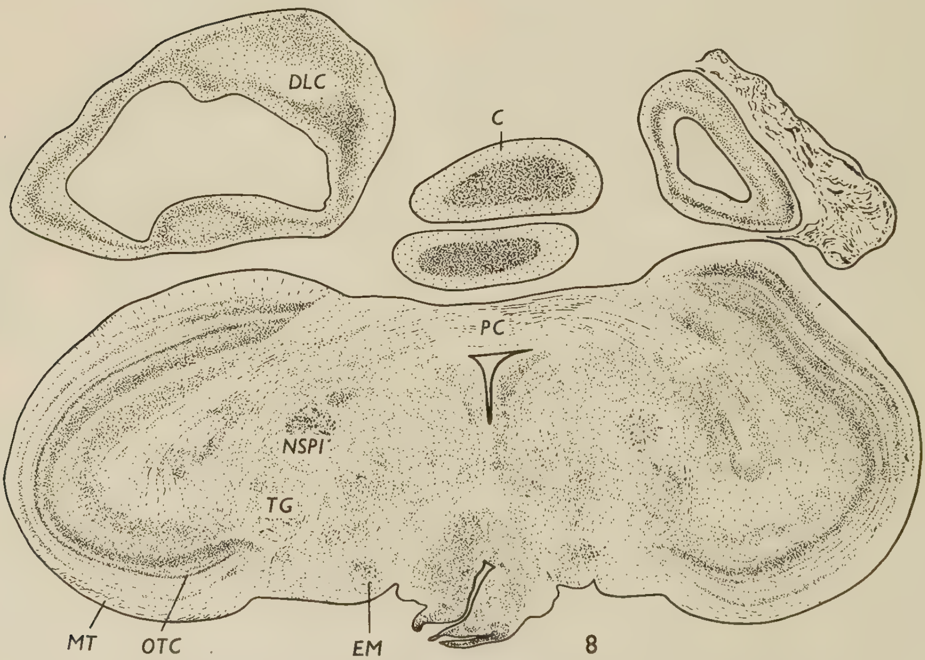
corresponding to the nucleus postrotundus of Huber & Crosby, 1929, but cf. Kappers *et al.* 1936) there is a marked reduction in cell size but no appreciable change in the amount of Nissl material in the cytoplasm. In the medial smaller-celled area (? the nucleus posterointermedialis of Huber & Crosby, 1929) there is only a slight cell shrinkage but again no change in staining intensity. There is no evidence of cell loss in either part of the cell mass. In the ill-defined area usually designated the nucleus posteroventralis some cell loss may have occurred, but the most striking change is the marked shrinkage and pallor of the cells throughout the nucleus (Pl. 4, figs. 8, 9). Perhaps the most unexpected observation in this experiment is the finding of marked degenerative changes in the area labelled TG in Text-fig. 8 which is to be identified as the subpretectal nucleus of Huber & Crosby (1929). These changes are in the form of partial cell loss, and in addition there is shrinkage of the surviving cells (Pl. 4, figs. 8, 9).

The third group of nuclei, which show no degenerative changes at all, includes all the remaining thalamic nuclei as described by Huber & Crosby (1929). A complete list of these nuclei need not be given, but it should be emphasized that this group contains such well-defined elements as the so-called lateral geniculate nucleus (Text-figs. 5–7), both parts of the nucleus spiriformis (Text-fig. 8), the medial and lateral habenular nuclei (Text-figs. 5, 7) and the nucleus of the habenulo-peduncular tract.

Experiment OP 40 (47 days' survival) is the most complete telencephalic ablation in our series; it has not been described more fully as representative of the first group of experiments because (1) there is some involvement of the dorsomedial cortex of the opposite hemisphere, (2) the involvement of the dorsolateral margin of the thalamus is somewhat greater than in OP 20, and (3) the oblique plane in which the sections have been cut make it difficult to compare the thalamic nuclei on the two sides. The only region of the telencephalon remaining in this experiment is the posterior part of the septum and the medial preoptic area. The extent of the thalamic degeneration is almost identical with that found in OP 20, the most noteworthy difference being the almost complete degeneration of nucleus dorsolateralis posterior; in this nucleus, as in the nucleus dorsolateralis anterior, there is marked cell loss and shrinkage of the few surviving cells. Furthermore, in nearly all the affected nuclei the cellular changes are more marked than in OP 20. In particular the nucleus dorsomedialis anterior shows more definite pallor and shrinkage of its constituent cells; in the nuclei ovoidalis and rotundus only very few cells persist, and the cells in the subpredectal nucleus have all undergone marked shrinkage.

The third experiment in this group, OP 30 (42 days' survival) is very similar to OP 20 both in the site and extent of the lesion and the resulting thalamic degeneration. In view of this only the significant differences need be described. The telencephalon has been completely removed with the exception of the cortex on the medial aspect of the hemisphere, the septum and the ventromedial part of the palaeostriatum around the inferior angle of the ventricle. It is difficult, however, to decide whether the afferent connexions of these areas are completely preserved or not, because immediately in front of the preoptic areas both forebrain bundles are directly involved. The septo-mesencephalic tract shows some gliosis but is by no means completely degenerated. The main differences in the thalamic degeneration





Text-figs. 7, 8. To show the caudal extent of the lesion in experiment OP 20 and the cell masses in the posterior part of the thalamus.

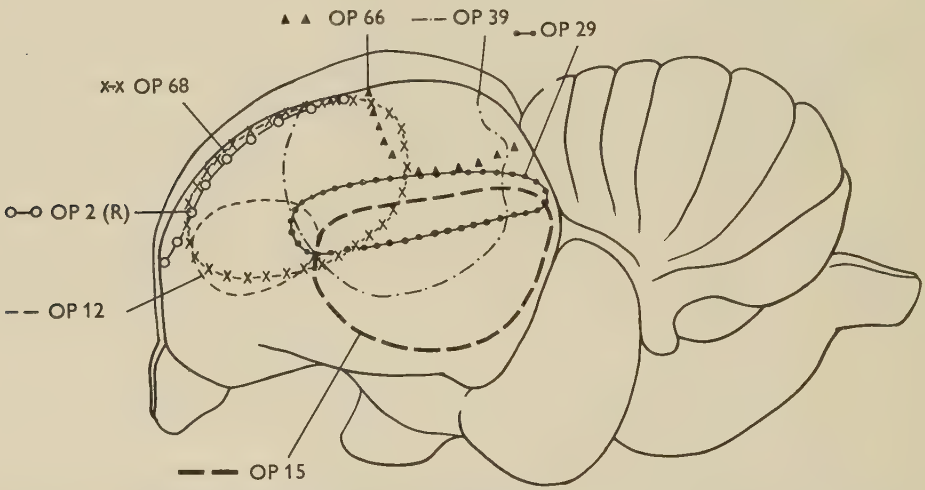
compared to experiment OP 20 are: in the nuclei ovoidalis, rotundus and dorsolateralis anterior the retrograde cell degeneration is even more severe and only an occasional pale-staining cell can be seen (Pl. 3, fig. 5). On the other hand, there is little change in the nucleus dorsomedialis and the degeneration in the subpretectal nucleus is less pronounced, the cells being distinctly paler than on the normal side, but they appear to be less shrunken than in OP 20.

Apart from minor differences in the degree of degeneration in the nuclei caudal to the nucleus rotundus and in the dorsomedial nucleus, the only significant difference in these three experiments is the finding of retrograde cell degeneration in the nucleus dorsolateralis posterior of OP 40. Unfortunately in that experiment the lesion had encroached upon the dorsolateral aspect of the diencephalon so that it is uncertain whether the degeneration may have been the result of this direct involvement. From the fourth experiment of this group, OP 2, however, this possibility can be excluded because here the nucleus dorsolateralis posterior has undergone degeneration after a lesion strictly limited to the telencephalon. With the exception of a very small portion of the ventromedial margin of the hemisphere the entire telencephalon has been destroyed. In the anterior one-third of the hemisphere only the prepyriform cortex and the immediately adjoining parts of the neostriatum and hyperstriatum around the inferior angle of the ventricle remain. In the succeeding sections the amount of surviving tissue can be seen to remain more or less constant, but at more posterior levels it is composed of the medial part of the palaeostriatum augmentatum on the lateral side of the ventricle and the ventral half of the cortex medial to the ventricle. This cortex is profoundly atrophied, particularly the molecular layer. It is probable that most of the striatum which remains is isolated by an extension of the lesion into the lateral forebrain bundle which severely disrupts its fibres. The anterior two-thirds of the septum are severely shrunken and partially involved, but the posterior one-third is largely preserved. The dorsolateral margin of the lateral preoptic area has been encroached upon, and the forebrain bundles directly involved. The septo-mesencephalic tract shows intense gliosis. Both the distribution and the severity of the retrograde degeneration in the thalamus are essentially the same as in the previous experiments, with the notable exception that both the anterior and posterior components of the nucleus dorsolateralis are severely affected. From this experiment it may be concluded that all the nuclei of the dorsal group are telencephalic dependencies. Although this experiment illustrates best the total thalamic projection, it has not been described as the primary example of a complete telencephalic ablation because there is some involvement of the medial aspect of the opposite hemisphere; this part of the lesion, however, has not resulted in any thalamic degeneration.

(II) *The organization of the thalamic projection*

Two observations described in the first section suggest that the individual elements of the avian thalamus, like the nuclei of the mammalian dorsal thalamus, have an organized projection upon the telencephalon. In the first place, it has been found that certain components of the dorsal nuclear group degenerated in some but not all experiments, and secondly, that the nature and degree of cellular degeneration differ in the various nuclear groups. In view of the remarkable structural dif-

ferentiation of both the telencephalon and the thalamus in the avian brain an attempt has been made to determine the projection of the individual thalamic nuclei. In the experiments presented in this section lesions varying in extent and distribution have resulted in differential involvement of those thalamic nuclei which have been shown to project upon the telencephalon. An analysis of this material has indicated that the efferent fibres from the thalamus terminate principally in two parts of the telencephalon: the palaeostriatum and the dorsomedial margin of the hemisphere. Further, the corollary of these findings, that a considerable portion of the hemisphere—including the dorsolateral cortex, the neostriatum and hyperstriatum—does not receive a projection from the thalamus, in the sense at least that their destruction does not result in retrograde cell degeneration in the thalamus, has also been demonstrated.



Text-fig. 9. Diagrammatic reconstruction of the superficial extent of the lesion in several experiments of the first group to show that together they cover the greater part of the dorsolateral aspect of the hemisphere.

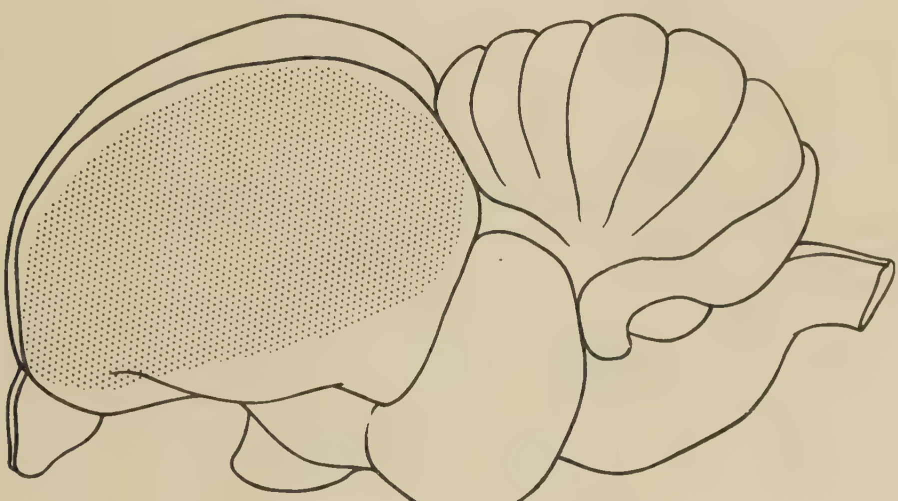
The experiments fall naturally into two classes: those in which the lesion has not resulted in retrograde cell degeneration, and those in which thalamic degeneration has occurred. Only representative examples of each class will be described in detail.

Experiments with no thalamic degeneration

There are thirteen hemispheres with superficial lesions of varying extent and in which no thalamic degeneration has been found. All the lesions are on the dorsolateral surface of the hemisphere and involve the dorsolateral cortex together with the immediately subjacent parts of the striatum. The distribution and extent of most of the lesions are shown in Text-fig. 9, from which it can be seen that although no single lesion has involved the entire dorsolateral cortex the lesions taken together cover almost the whole area. The largest lesion may be taken as representative of this group and will be described in some detail.

In experiment OP 9 (survival period 62 days) an extensive superficial lesion has

destroyed most of the dorsolateral surface of the left hemisphere throughout its antero-posterior extent (Text-fig. 10). The lesion begins at the frontal pole and gradually increases in extent and depth until it is maximal at the level of the appearance of the palaeostriatum. Thereafter the extent of the damage diminishes —particularly in depth—back to the level of the posterior end of the septum, after which it remains constant. The entire dorsolateral cortex of this hemisphere is completely destroyed, and the prepyriform and parentorhinal areas are encroached upon slightly. The lateral margin of the accessory hyperstriatum has been involved, together with the dorsal part of the hyperstriatum and neostriatum frontale and intermediale. The neostriatum caudale has suffered slight damage along its ventricular margin, and at these levels the dorsolateral part of the archistriatum has also been encroached upon (Text-fig. 11).



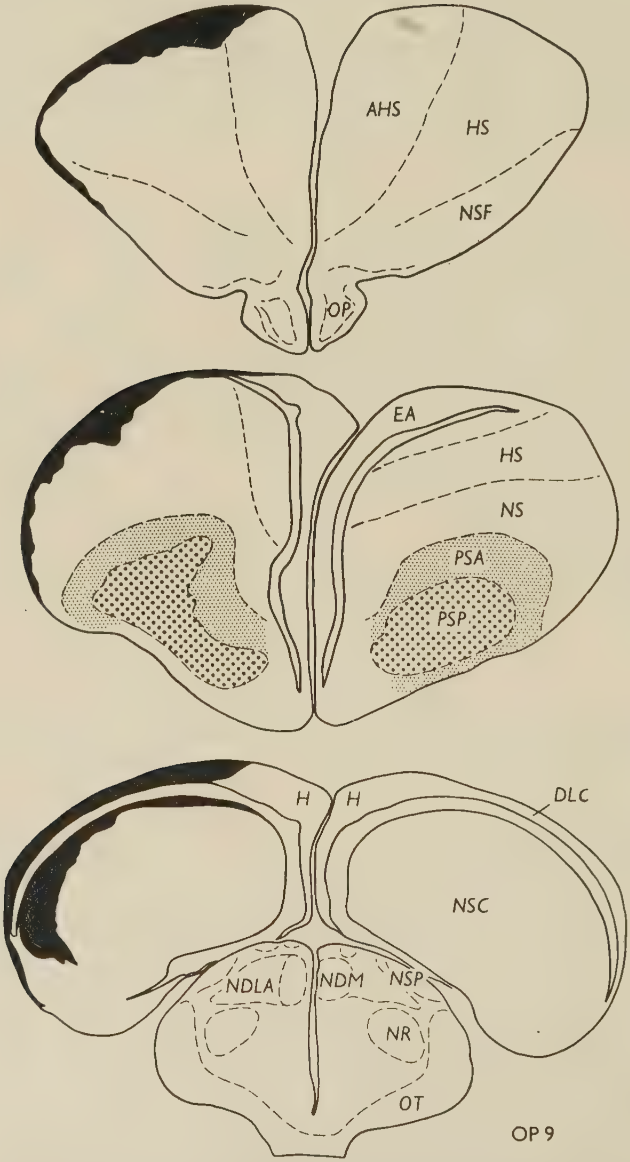
Text-fig. 10. Diagram to show the extent of the lesion (stippled) as projected upon the surface of the brain in experiment OP 9.

Experiments with thalamic degeneration

From an analysis of the remaining experiments with smaller localized lesions it soon becomes apparent that they can be divided into two groups on the basis of the distribution of the thalamic degeneration. In the first group only the dorsal nuclei and the nucleus superficialis parvocellularis degenerate together or individually, while in the second the degeneration also involves the nuclei rotundus, subrotundus and ovoidalis. Examples of each of these two groups will first be described before presenting the experimental evidence for the localization of the projection of individual nuclei.

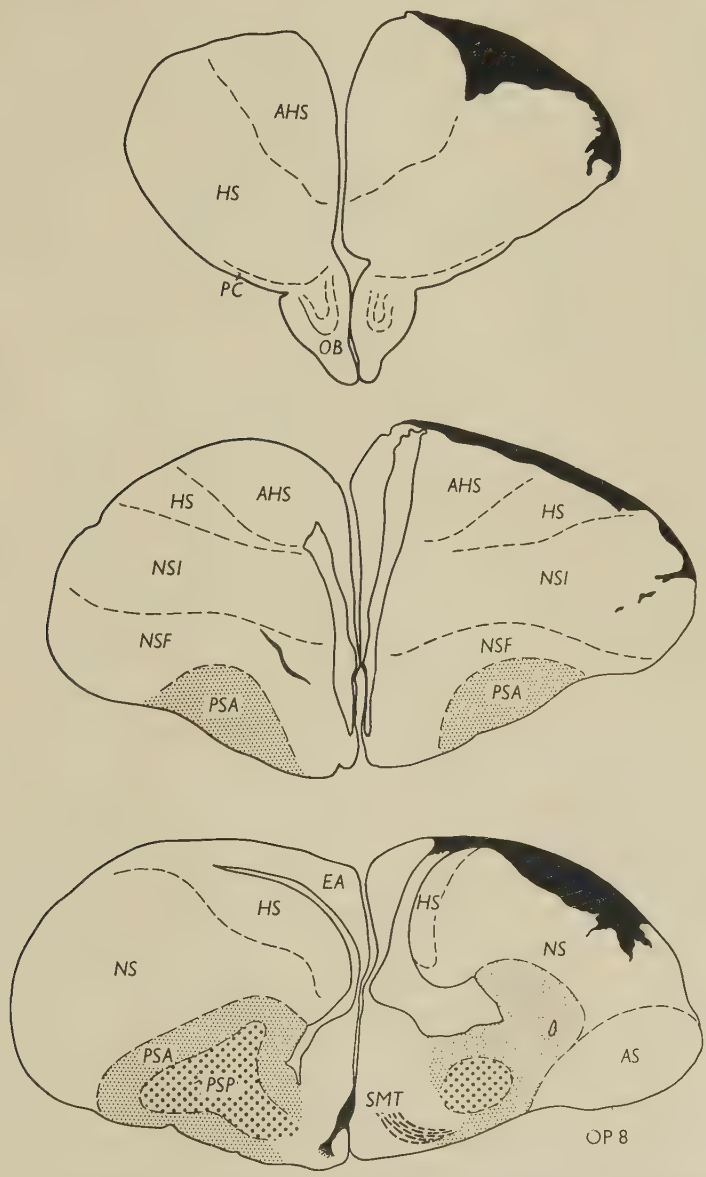
In the first experiment of the first group, OP 8, a large lesion which has involved the dorsal striatal areas of the right hemisphere has resulted in degeneration only in the nucleus dorsolateralis anterior and nucleus superficialis parvocellularis. The lesion begins at the frontal pole where it involves the lateral margin of the accessory

hyperstriatum and the dorsal part of the hyperstriatum. More caudally it comes to involve the dorsal surface of the neostriatum with no additional involvement of the accessory hyperstriatum. Virtually the whole dorsolateral cortex and the dorsal part of the neostriatum caudale have been destroyed almost to the caudal pole of the hemisphere. Medially the lesion does not extend beyond the lateral ventricle and at no point does it encroach upon the palaeostriatum or the archistriatum (Text-fig. 12).



Text-fig. 11. The extent of the lesion in the left hemisphere in experiment OP 9 at three antero-posterior levels through the hemispheres. The damaged areas in this and the subsequent figures are indicated in black.

In the thalamus definite cellular degeneration is seen in the nucleus dorsolateralis anterior in the form of a moderate degree of cell loss and gliosis together with pallor and shrinkage of all the remaining cells. In the nucleus superficialis parvocellularis



Text-fig. 12. The extent of the damage to the two hemispheres in OP 8. The small lesion in the left hemisphere has completely interrupted the fibres of the septo-mesencephalic tract.

there is some cell shrinkage but the principal change is the pallor of the cells. It should be emphasized that no other thalamic nucleus shows evidence of retrograde degeneration and in particular that the entopeduncular nucleus is unaffected.

In experiment OP 7 the lesion is considerably smaller in its anteroposterior extent but involves rather more of the accessory hyperstriatum. It commences at the frontal pole of the hemisphere, destroying the whole of the accessory hyperstriatum and the dorsal part of the hyperstriatum. The extent of the damage remains more or less constant through the anterior third of the hemisphere except for a narrow knife-cut into the lateral part of the neostriatum intermediale. The entorhinal area and dorsolateral cortex are not involved. The lesion diminishes in size relatively suddenly and ends just in front of the appearance of the septum.

The changes in the nuclei dorsolateralis anterior and superficialis parvocellularis are essentially the same as those described for experiment OP 8, but in addition there are unequivocal changes in the nucleus dorsomedialis anterior. Here there is a distinct pallor of all the cells and many are unmistakably shrunken. Again there is no evidence of degeneration in any of the other thalamic nuclei, including the nucleus entopeduncularis.

In the remaining experiments of this group with comparable lesions no degeneration has been found in nuclei other than those of the dorsal group. On the other hand, in a larger group of experiments in all of which there has been some involvement of the palaeostriatum, changes have been consistently found in the nucleus rotundus and/or the nucleus ovoidalis, together with varying degrees of degeneration in the other nuclei which show changes after the large telencephalic lesions described in the previous section. That the critical factor leading to degeneration in these nuclei is in fact involvement of the palaeostriatum is shown in the following two experiments which are representative of this second group.

In experiment OP 6 the lesion has involved both hemispheres but to a varying extent and with significantly different degeneration in the thalamus of the two sides. On the right side most of the damage is in the anterior one-third or half of the hemisphere beginning close to the anterior pole where there is a superficial area of damage in the accessory hyperstriatum. The lesion rapidly increases in size to destroy completely the accessory hyperstriatum, the hyperstriatum and neostriatum frontale. At the level at which the palaeostriatum is first seen the lateral part of the neostriatum and hyperstriatum re-appear so that the lesion is now restricted to the medial part of the striatum. Behind this level the lesion becomes progressively smaller in size and extends back as a central core of damage close to the lateral ventricle damaging the medial parts of the hyperstriatum and neostriatum. The damage ceases completely just in front of the level of appearance of the septum. On this side the only damage to the palaeostriatum is some slight involvement of its dorsolateral margin at anterior levels.

On the left side the damage also begins at the frontal pole as a superficial area of destruction in the dorsolateral part of the hemisphere involving only the lateral portion of the accessory hyperstriatum and the dorsal hyperstriatum. It remains more or less constant in size, and although it comes to involve the lateral part of the neostriatum the damage to the accessory hyperstriatum remains minimal. In the anterior third of its extent the palaeostriatum is not involved, but in the sudden increase in the size of the lesion, which occurs just rostral to the septum, its dorsolateral margin together with the lateral two-thirds of the neostriatum and the hyperstriatum are destroyed. At the level of appearance of the septum the entire

hyperstriatum, neostriatum and palaeostriatum augmentatum are destroyed, but the accessory hyperstriatum and entorhinal areas are intact. At about the middle of the septum the lesion diminishes in size and becomes progressively restricted to the dorsomedial parts of the hyperstriatum and the neostriatum, the adjoining dorsal part of the palaeostriatum and to a narrow extension of the lesion which reaches ventrally through the middle of the palaeostriatum just rostral to the preoptic areas. Behind this the lesion extends back almost to the caudal pole of the hemisphere in the medial part of the neostriatum caudale: the overlying dorsolateral cortex is undamaged. The archistriatum has not been directly involved by the lesion but has undergone ischaemic necrosis with virtually complete cell loss.

On the right side (i.e. the side with the smaller lesion) there is pronounced gliosis throughout the cross-sectional area of the septo-mesencephalic tract. Beginning anteriorly in the considerably shrunken cortex on the medial side of the ventricle, this gliosis can be seen on succeeding sections to pass caudally and ventrally, to the dorsomedial border of the septum. From here it can be followed as it passes downwards through the medial part of the septum and then ventrally in a course reminiscent of that of the diagonal band of the mammalian brain. It reaches the ventral surface of the hemisphere medial to the forebrain bundles and then sweeps laterally beneath these fibre bundles to the lateral surface of the diencephalon. Hence it passes dorsally around the lateral aspect of the thalamus in front of the optic tract to reach, finally, the region of the nucleus superficialis parvocellularis. On the other side, despite the larger lesion, there is only slight gliosis in the septo-mesencephalic tract, and there is a striking difference in the thickness of the medial wall of the hemisphere on the two sides.

In the thalamus of the right side unequivocal retrograde degeneration has occurred in the nuclei dorsolateralis anterior and superficialis parvocellularis and in the anterodorsal part of the nucleus rotundus. The most anterior part of the nucleus dorsolateralis anterior and, at more posterior levels, the dorsomedial part of the nucleus, are intact but elsewhere the nucleus shows marked cell loss and gliosis. The cells of the nucleus superficialis parvocellularis are distinctly shrunken and pale-staining. In the affected part of the nucleus rotundus there is marked cell loss and pallor of the surviving cells. In addition to these changes there is a suggestion of cell shrinkage in the dorsomedial nucleus at anterior levels and there is some shrinkage of the cells in the lateral part of the entopeduncular nucleus. On the left side, the thalamus shows severe retrograde change in the nuclei ovoidalis, rotundus and subrotundus. In each of these nuclei there is an almost total cell loss with marked shrinkage and pallor of the few remaining cells. Other nuclei which are affected are the entopeduncular, the dorsolateral anterior and posterior, the superficialis parvocellularis, the postrotundus and the posteroventral. The changes in the dorsal nuclei are comparable qualitatively to those in the opposite thalamus, but are restricted to the lateral parts of the nuclei; the changes in the nuclei postrotundus and posteroventral are difficult to assess in view of the obliquity of the sections, but in both nuclei there appears to be a moderate degree of cell shrinkage. The cells of both elements of the entopeduncular nucleus are shrunken and pale-staining, especially in the dorsal part of the nucleus.

The interest of this experiment lies in the fact that the lesion on the two sides and

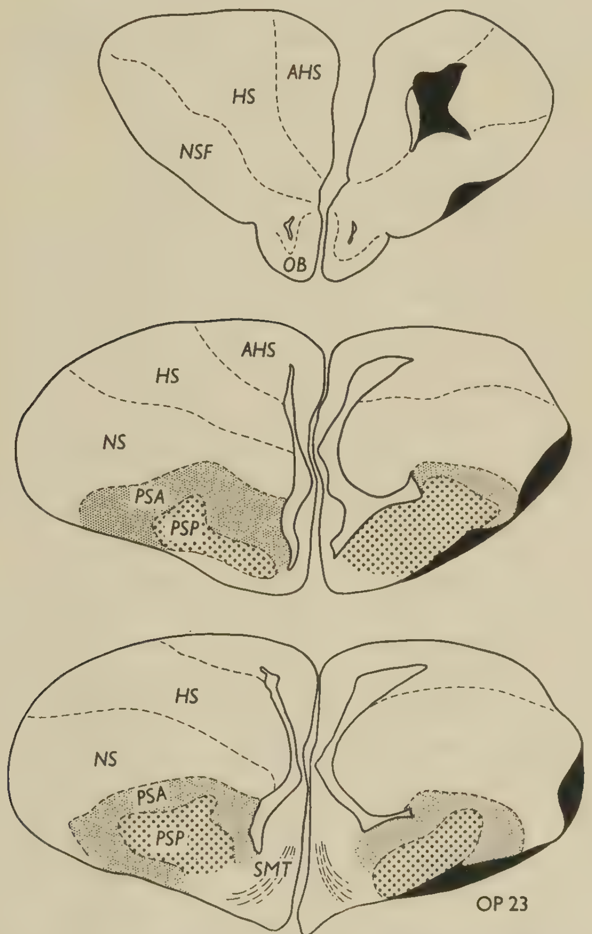
the resulting thalamic degeneration are almost complementary to each other. On the right side the lesion essentially destroys the medial part of the anterior third of the hemisphere with only minimal involvement of the palaeostriatum, while on the left side it is principally the dorsolateral and central portions of the hemisphere which are involved, with considerable damage to the palaeostriatum. The thalamic degeneration on the two sides is correspondingly different: on the right side with the smaller lesion, only the nuclei of the dorsal group and a small part of the nucleus rotundus have been affected, while on the opposite side the nuclei ovoidalis, rotundus and subrotundus are predominantly affected and slight changes are present in the dorsal group. A correlation of the findings in this experiment with those of OP 9, the representative of the second group in which no thalamic degeneration occurs after a large, but relatively superficial lesion, suggests that involvement of the palaeostriatum is the critical factor determining whether or not degeneration occurs in the thalamic nuclei (other than those of the dorsal group) which degenerate after complete removal of the telencephalon. That the palaeostriatum is the critical factor is further exemplified by experiment OP 23 in which, after a relatively small lesion involving the lateral part of the palaeostriatum, marked retrograde degeneration has occurred in the nucleus rotundus.

The lesion in this experiment (Text-fig. 13) begins close to the frontal pole but for the greater part of its extent is confined to the ventrolateral quadrant of the hemisphere. Anteriorly, the medial third of the hyperstriatum has been destroyed, together with the ventrolateral margin of the neostriatum frontale. The lesion remains more or less constant back to the level of appearance of the palaeostriatum behind which only the lateral margin of this structure and the lateral part of the neostriatum intermediale are affected. The palaeostriatum has been directly involved ventrolaterally; the ventral third of the palaeostriatum augmentatum has been completely removed, and there is a narrow extension of the lesion which passes vertically through the primitivum. Although not directly affected, a large part of the medial end of the palaeostriatum augmentatum shows a distinct loss of cells, probably due to vascular involvement. The damage to the palaeostriatum is maximal at the level of the anterior end of the septum, but it diminishes rapidly in extent to cease well in front of the posterior end of the septum. At this level the damage to the neostriatum is also considerably reduced, involving only its lateral margin. Distinct gliosis, continuous with the lesion in the palaeostriatum, can be traced back in the lateral forebrain bundle, but it should be emphasized that the dorsomedial part of the hemisphere, including the accessory hyperstriatum, has not been involved at all, and that there is no gliosis in the septo-mesencephalic tract.

The degeneration in the thalamus is surprisingly extensive in view of the restricted nature of the lesion. The anterior two-thirds of the nucleus rotundus is completely devoid of cells, but at the junction of its middle and posterior thirds a few normal cells appear and progressively increase in number until the posterior limit of the nucleus appears almost normal. In the posteroventral and postrotundus nuclei and in the subpretectal nucleus the cells show marked shrinkage comparable to that seen after the complete telencephalic ablations. Likewise, the dorsal part of the entopeduncular nucleus is severely affected, especially anteriorly; here there is a very severe cell shrinkage and gliosis while in the ventral part of the nucleus there is

some degree of pallor of the cells, no distinct cell shrinkage but an intense gliosis. The nuclei of the dorsal group and the nuclei ovoidalis and subrotundus, on the other hand, are completely unaffected (Pl. 5, fig. 10).

These two experiments, taken together, indicate that not only do the telencephalic-dependent nuclei have a differential projection upon the cerebral hemisphere, but also that within the projection of individual nuclei there is at least some degree of



Text-fig. 13. To show the extent of the lesion in the right hemisphere of experiment OP 23 at three anteroposterior levels.

topical organization. Thus from experiment OP 23 it is apparent, for example, that the nucleus rotundus may degenerate independently of the nucleus ovoidalis, and furthermore that within the projection of the nucleus rotundus there is a definite anteroposterior organization in so far as the degeneration in the nucleus is confined to its anterior two-thirds. Similarly, on the side of the smaller lesion in OP 6 the finding of degeneration confined to the dorsomedial part of the nucleus rotundus

suggests a comparable mediolateral organization of the efferent connexions of this nucleus. An additional conclusion which may be drawn from these two experiments is that there is some correlation between the site and the extent of the lesion in the palaeostriatum and the distribution of the resulting thalamic degeneration.

The purpose of presenting the results of several of the remaining experiments of this group in some detail is to examine the validity of these hypotheses. It will be convenient to present the evidence for the localization of the projection of the nucleus ovoidalis and the rotundus before dealing with the nuclei of the dorsal group. The lesions of the remaining experiments will not be described in full, but for each of these two nuclear groups only the relevant damage to the palaeostriatum and the dorsomedial aspect of the hemisphere, respectively, need be described.

In experiment OP 26 there is extensive damage to the hyperstriatum, neostriatum and dorsolateral cortex and at the level of appearance of the palaeostriatum primitivum the lateral third of the palaeostriatum augmentatum has been destroyed. From this level the lesion extends caudally, destroying approximately the same amount of the palaeostriatum augmentatum and the lateral margin of the palaeostriatum primitivum back to the level of the anterior commissure. Here the most ventral part of the lesion encroaches upon the lateral aspect of the lateral forebrain bundle.

In the thalamus there is a complete cell loss in the nucleus rotundus throughout its anteroposterior extent. In the nuclei ovoidalis and subrotundus there is definitely no cell loss, but there is a suggestion of cell shrinkage and pallor. There are also changes in the nuclei entopeduncularis, posteroventralis, postrotundus and the subpretectal nucleus in the form of cell shrinkage; the nuclei of the dorsal group are unaffected.

This experiment differs from the previous experiment in two respects. First, the lesion involves considerably more of the lateral palaeostriatum and extends throughout its anteroposterior extent; secondly, the degeneration in the nucleus rotundus is correspondingly more extensive. However, despite these differences they have the important common feature that the nucleus rotundus has degenerated independently of the nucleus ovoidalis. The next experiment to be described is virtually complementary to these two experiments. Here the nucleus ovoidalis has degenerated while the nucleus rotundus is unaffected. As the precise projection of the entopeduncular nucleus and of the nuclei posterior to the rotundus has not been determined, and as the organization of the projection of the dorsal nuclei will be treated separately, the cellular changes in these nuclei will not be described in the subsequent experiments dealing with the projection of the nuclei ovoidalis and rotundus.

In the brain of pigeon OP 5, lesions have been placed in both hemispheres, but the smaller lesion in the right side has not resulted in any thalamic degeneration and will not be described here. The lesion on the left side extends throughout the anteroposterior extent of the hemisphere, damaging the anterior part of the accessory hyperstriatum, most of the dorsal hyperstriatum, large areas of all parts of the neostriatum and the dorsolateral cortex. The involvement of the palaeostriatum begins at the level of the appearance of the palaeostriatum primitivum where the dorsal and medial parts of the palaeostriatum augmentatum are destroyed. Back to the level of the anterior commissure the only additional damage to the palaeo-

striatum is to the dorsal and medial portion of the palaeostriatum primitivum, the lateral part of the palaeostriatum augmentatum remaining intact. It is difficult to be certain, but it is probable, that in this experiment almost the entire ektostriatum has either been severely damaged or completely removed; certainly the only portion which might be spared is the medial part immediately adjoining the palaeostriatum.

In the thalamus there is very marked cellular degeneration in the nucleus ovoidalis while the nucleus rotundus is largely preserved. The changes in the nucleus ovoidalis are in the form of severe cell loss with shrinkage and pallor of all the surviving cells. In the nucleus rotundus, on the other hand, the degenerative changes are confined to the posterior third of its anteroposterior extent where the majority of the cells are shrunken, but there does not appear to be any cell loss. The nucleus subrotundus similarly shows cell shrinkage rather than cell loss. It is of particulr interest that in this experiment the entopeduncular nucleus shows shrinkage and pallor of the cells only in the medial portion of the nucleus which is surrounded by the intense gliosis in the lateral forebrain bundle.

The significance of this experiment is twofold; in the first place it shows that the nucleus ovoidalis can undergo retrograde degeneration independently of the rotundus and thus has a separate projection field; and secondly, it confirms the findings of the previous experiments that the nucleus rotundus is related to the lateral part of the palaeostriatum. In addition it probably excludes the ektostriatum as the main site of termination of the axons from rotundus since it shows only slight changes after virtually complete destruction of the ektostriatum (cf. Huber & Crosby, 1929).

Experiment OP 70 provides further confirmation on these two points. In this case the lesion is essentially in two parts: a large superficial lesion destroying the dorsal and lateral aspects of the hemisphere and a narrow knife-track on the medial side of the hemisphere which extends down parallel to the ventricle into the palaeostriatum. A wedge-shaped medial extension of the superficial lesion has also encroached upon the lateral aspect of the palaeostriatum and has resulted in necrosis of the central third of its anteroposterior extent. The other palaeostriatal lesion has destroyed the dorsal aspect of the palaeostriatum augmentatum immediately beneath the damaged neostriatum intermediale. This latter involvement of the palaeostriatum is confined to the posterior third of its extent.

The thalamic degeneration in this case resembles that found in the previous experiment. The nucleus ovoidalis is severely degenerate throughout its extent, but the nucleus rotundus shows cellular degeneration only in its dorsal part. The nucleus subrotundus, however, is not nearly so severely affected and in fact only shows shrinkage and pallor of the cells in its medial part.

In experiment OP 62 the lesion is confined to the posterior half of the hemisphere. In addition to damaging the dorsal and lateral parts of the hyperstriatum and neostriatum it has extended into the lateral ventricle to destroy the posteromedial part of the neostriatum caudale and the adjacent dorsomedial part of the palaeostriatum—principally the medial third of the palaeostriatum augmentatum. In the thalamus of this experiment the most striking cellular degeneration is seen in the nucleus ovoidalis; here there is a very severe cell loss with pronounced shrinkage

and pallor of the few remaining cells and an intense gliosis. The nuclei rotundus and subrotundus are completely unaffected.

Taken together, these experiments with degeneration only in the nucleus ovoidalis, or largely confined to that nucleus, clearly indicate that its projection field is the medial part of palaeostriatum. Furthermore, since degeneration in the nucleus is always most severe with lesions in the posterior part of the striatum it would appear that either the majority of the projection fibres terminate in the posterior part of the palaeostriatum or at least pass through it to reach more anterior levels. That this conclusion is substantially correct is shown by the next experiment, OP 13, in which a lesion in the anteromedial part of the palaeostriatum has resulted in degeneration in the nucleus ovoidalis which is qualitatively different from that seen in the previous experiments.

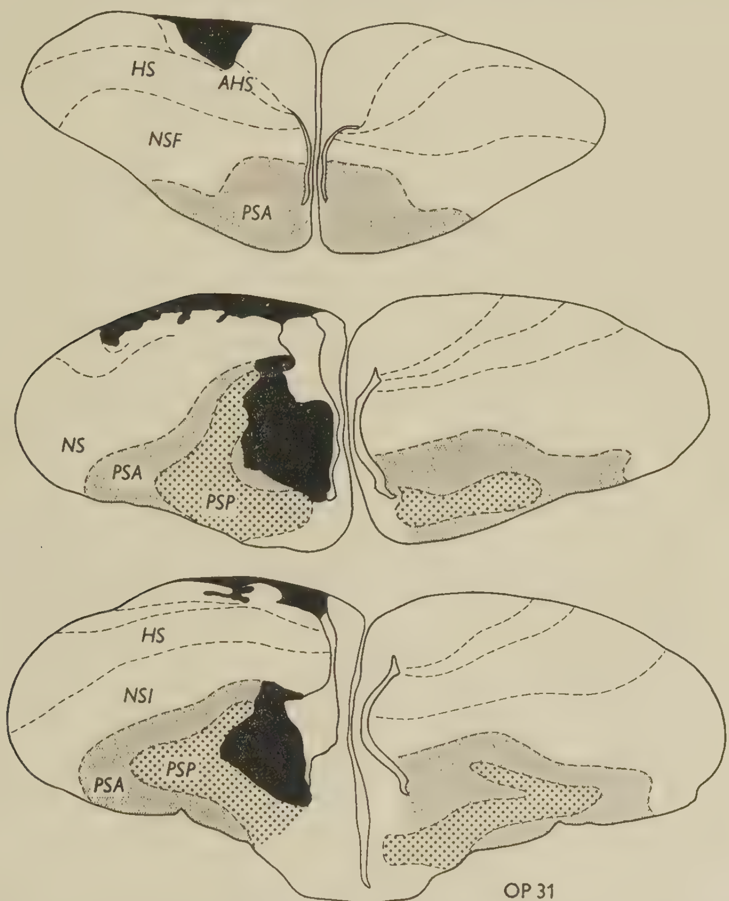
The damage to the palaeostriatum in OP 13 is limited to the anterior two-thirds of the medial part of the palaeostriatum augmentatum with two narrow extensions into the medial part of the palaeostriatum primitivum. In the thalamus there is again no change in the nuclei rotundus and subrotundus, but there is marked cell shrinkage in the ovoidalis. Under a low-power objective the degeneration appears to be about as severe as in previous experiments, but at higher magnifications it is apparent that there is no cell loss in the nucleus but that the majority of the cells are distinctly shrunken and pale staining.

The projection of the nucleus rotundus to the palaeostriatum augmentatum rather than to the palaeostriatum primitivum is shown by experiment OP 23: that this is true also for the nucleus ovoidalis is demonstrated by experiment OP 31. In this case the nucleus ovoidalis has undergone severe retrograde degeneration after a lesion which at no point encroaches upon the palaeostriatum primitivum. The definitive part of the lesion has directly damaged the dorsal part of the palaeostriatum augmentatum over a very short distance but has also resulted in a fairly extensive area of ischaemic necrosis in the posterior half of the medial sector of the palaeostriatum augmentatum (Text-fig. 14). As far as the thalamus is concerned it need only be noted that here again the nucleus ovoidalis is severely affected without concomitant changes in the nuclei rotundus and subrotundus (Pl. 5, fig. 11).

The accumulated evidence of the above experiments seems to establish beyond doubt that the nuclei ovoidalis and rotundus project independently, that they are connected with the palaeostriatum augmentatum and that the nucleus rotundus is related to its lateral part while the ovoidalis is connected with its medial part. However, these experiments have left undecided the precise projection of the nucleus subrotundus, although they have shown that like the other nuclei it has an independent projection. For example, it has been shown that the nucleus may degenerate with one or both of the other two central nuclei, and that it is not infrequently preserved when the others are degenerated. One final experiment, OP 21, clearly establishes the independence of the projection of the nucleus subrotundus for in this brain this nucleus shows virtually no change after a lesion which has resulted in severe degeneration of the rotundus and ovoidalis.

The damage to the palaeostriatum in this experiment extends throughout its posterior two-thirds, destroying most of the palaeostriatum augmentatum and the dorsal and lateral parts of the primitivum. The most anterior part of the palaeo-

striatum augmentatum has escaped injury, but from the level of appearance of the palaeostriatum primitivum back to the caudal limit of the palaeostriatum only the medial margin of the palaeostriatum augmentatum is unaffected. The nuclei rotundus and ovoidalis are both completely degenerated, but the nucleus subrotundus stands out in striking contrast to these elements since, at the most, it shows only slight cell shrinkage and there is certainly no cell loss in the nucleus (Pl. 6, fig. 12). When considered together with the previous experiments the conclusion to



Text-fig. 14. Representative sections through the lesion in experiment OP 31. The black area in the medial part of the palaeostriatum represents the site of the critical damage which has resulted from ischaemic necrosis.

be drawn from this experiment is that the nucleus subrotundus either projects to more anterior parts of the palaeostriatum augmentatum or more ventrally to the palaeostriatum primitivum.

It has been mentioned above that the precise projection of the nuclei posterior to the nucleus rotundus, viz. the postrotundus, the posteroventral and the subpretectal nucleus, cannot be determined from the present material. Indeed the material

available is insufficient to establish whether or not these nuclei have a projection independent of each other or of the three central nuclei; that they are independent of the nuclei of the dorsal group, however, has been demonstrated by experiments OP 7 and OP 8 described above. The fact that they so frequently show changes which parallel the degeneration in the nuclei rotundus and ovoidalis would suggest that they either project directly to the palaeostriatum augmentatum or that they are in some way connected with the projection of the central nuclei. Similarly, the constant finding of degeneration in the entopeduncular nucleus following lesions of the palaeostriatum strongly suggests that this nucleus is closely related to the projection of the central group of nuclei. The findings in OP 5, in which degeneration has been found only in the medial half of the nucleus, would also suggest that there is a mediolateral organization of its projection fibres comparable to that found in the nuclei ovoidalis and rotundus.

A similar series of experiments will now be described in an attempt to define the projection area of the individual elements of the dorsal nuclear group. The first experiment to be considered, OP 44, serves to delimit, in a negative way, the projection field of these nuclei as it has the largest lesion of the series in which no degeneration has occurred in these nuclei. The lesion begins immediately behind the frontal pole of the hemisphere where there is a small area of damage in the most dorsal part of the hyperstriatum. Behind this the hyperstriatum is increasingly involved and the accessory hyperstriatum is encroached upon. At the level of appearance of the palaeostriatum the hyperstriatum is completely destroyed together with the dorsal half of the neostriatum frontale. In addition there is a cut extending down through the lateral part of the accessory hyperstriatum into the lateral ventricle. With the appearance of the palaeostriatum primitivum the lesion increases in size to include the whole of the hyperstriatum, neostriatum and the lateral part of the palaeostriatum augmentatum. The cortex on the medial wall of the hemisphere is preserved. From the anterior end of the septum back to the level of the anterior commissure the only structures not completely destroyed are the ventral two-thirds of the palaeostriatum primitivum, the inferolateral part of the neostriatum and the cortex medial to the lateral ventricle. More caudally the lesion destroys the middle portion of the hemisphere as far as the caudal pole, only the most ventral part of the archistriatum, the lateral part of the neostriatum caudale and the medial cortical area being preserved.

Despite the fact that this extensive lesion has resulted in severe retrograde degeneration in the nuclei ovoidalis, rotundus, subrotundus and the nuclei of the posterior group no appreciable change has occurred in any of the dorsal nuclei. In this respect this experiment is virtually complementary to experiments OP 7 and OP 8 in which the dorsal nuclei degenerated independently of the others. Together these three experiments limit the area of projection of the dorsal nuclei to the dorsomedial margin of the hemisphere, to either the cortex in this area or to the accessory hyperstriatum. A second interesting feature in OP 44 is the absence of gliosis in the septo-mesencephalic tract. This stands in marked contrast to the severe glial proliferation which has occurred in the lateral forebrain bundle. The significance of this absence of gliosis in the septo-mesencephalic tract is apparent when one compares this experiment with, say, the findings in experiment OP 6 in which this tract was

severely atrophied and the dorsal nuclei completely degenerated. Indeed, it has been an invariable finding that in all experiments in which degeneration of the dorsal nuclei has occurred there is an accompanying gliosis in the septo-mesencephalic tract. This is emphasized by the next experiment in which division of the septo-mesencephalic tract has resulted in selective degeneration of the dorsal nuclei.

In the left hemisphere of OP 8 there is a barely detectable needle track which passes downwards and backwards through the medial parts of the accessory and dorsal hyperstriatum, neostriatum and palaeostriatum to reach the ventromedial angle of the hemisphere below the septum. Here there is a small focal lesion less than 1 mm. in diameter which has completely interrupted the fibres of the septo-mesencephalic tract, but has caused only minimal damage to the adjacent parts of the septum and medial preoptic areas (Text-fig. 4). Extending forwards and backwards from this lesion there is intense gliosis in the septo-mesencephalic tract which follows the course already described in OP 6. The gliosis can be traced in the molecular layer of the cortex medial to the ventricle as far dorsally as the superior angle of the ventricle and anteriorly to the level of the accessory hyperstriatum. In the thalamus of this side all the dorsal nuclei show degenerative changes. In the nucleus dorsomedialis the majority of the cells are shrunken and pale staining; there is comparable pallor of the cells of the nucleus superficialis parvocellularis; in the nuclei dorsolateralis anterior and posterior there is some degree of cell loss and quite marked shrinkage of the cells particularly in the posterior part of the nucleus. No other thalamic nuclei are affected. It may be concluded therefore that the septo-mesencephalic tract is the principal projection pathway of the dorsal nuclear group of the thalamus, whereas the other nuclei projecting upon the telencephalon send their fibres into the forebrain bundles.

The precise termination of the projection fibres in the septo-mesencephalic tract cannot be determined from our material. Examples have already been given, however, which indicate that not only is the projection independent of that of the central group of thalamic nuclei, but also that the different elements in the dorsal nuclear group project independently of each other. Thus in experiment OP 20, described in the previous section, the nucleus dorsolateralis anterior is severely degenerated while the dorsolateralis posterior remains unaffected. Similarly, in experiment OP 7 of the present series, the nucleus dorsomedialis anterior shows unequivocal retrograde degeneration, while on the side of the larger lesion in OP 8 this nucleus remains intact although all the other elements in the dorsal nuclear group are severely affected. In all the experiments so far described the lesions have been relatively large, and the resulting degeneration has always affected more than one of the dorsal nuclei. There are four brains in which degeneration is confined to only one of the dorsal nuclei after relatively small lesions in the anterior third of the hemisphere. Experiment OP 3 will be described as an example of this group.

In this brain the lesion begins at the frontal pole of the hemisphere where the dorsal half of the accessory hyperstriatum and rather less of the dorsal hyperstriatum are destroyed together with the entorhinal cortex. The lesion remains confined to the dorsomedial portion of the hemisphere, completely destroying the posterior part of the accessory hyperstriatum, the dorsal half of the entorhinal area and the dorsal third of the dorsal hyperstriatum. The cortex medial to the lateral

ventricle is preserved. Behind this the lesion becomes progressively smaller in size, destroying the dorsolateral cortex, the dorsal margin of the hyperstriatum and the lateral part of the entorhinal area. At the level of the septum it is confined to the dorsolateral and entorhinal cortical areas. In the thalamus the only nucleus which shows evidence of retrograde cell degeneration is the nucleus dorsolateralis posterior. Here there is a severe cell loss with marked shrinkage and pallor of the remaining cells and a moderately intense gliosis.

DISCUSSION

The surprising ease with which lesions could be placed in the brain of the pigeon and its remarkable ability to survive after quite extensive lesions have already been commented upon, but an equally unexpected finding is the strikingly clear-cut degenerative changes which can be found in the thalamic nuclei. This clarity of the degeneration may be attributed to two factors: first, to the very marked differentiation of most of the thalamic nuclei, and secondly, to the profound degree of cell change and gliosis which is found in those nuclei which project upon the telencephalon. The essential similarity of retrograde cell degeneration in the avian pontine homologue to that found in the pontine nuclei of mammals has been commented upon by Brodal, Kristiansen & Jansen (1950). These observations are in striking contrast to the findings of Powell & Kruger (1960) in a comparable study of the thalamic projection in the reptilian brain where the thalamic nuclei are poorly defined, and the cellular degeneration not at all marked, there being a noteworthy absence of gliosis. Indeed, in our experience, the retrograde degeneration found in the avian brain is as conspicuous as that seen in the mammalian thalamus after comparable survival periods.

The severity of the degeneration after complete removal of the telencephalon is by no means the same in all the affected thalamic nuclei so that it is possible to classify the nuclei on the basis of their reaction to telencephalic removal into two groups. In the first group are included those nuclei which undergo complete cell loss; in the second group are those nuclei which show unequivocal changes varying in degree from slight cell pallor to marked cell shrinkage and partial cell loss. The thalamic nuclei which show no change after total removal of the telencephalon may be considered as constituting a third group. Before discussing into which of these groups individual thalamic nuclei should be placed it is necessary to discuss briefly the possible significance of the different cellular reactions. The simplest explanation of these findings, suggested by analogy with the mammalian thalamus, is that all the affected nuclei send their axons to the telencephalon, and conversely those nuclei which show no change have no such telencephalic projection. The evidence upon which this explanation rests need not be discussed here as it has been fully reviewed for the mammalian thalamus by Walker (1938) and Rose & Woolsey (1943). An alternative interpretation which attempts to explain the specific differences in the degree of cell change between different nuclei is that only those nuclei which show complete cell loss (e.g. the nucleus rotundus) project exclusively upon the telencephalon. The less severe changes in other nuclei would then be explicable on the basis that they either give off collaterals to other diencephalic or brainstem structures which are capable of partially maintaining the integrity of the cell, or that the

degeneration in these nuclei is not due to a direct telencephalic projection but is secondary to the complete degeneration in nuclei like the rotundus. A third possibility which has to be considered is that some or all of the cellular changes in the thalamus are the manifestations not of retrograde degeneration but of transneuronal degeneration. That is to say, they have resulted not from axonal section but as a direct consequence of removal of descending connexions from the telencephalon. With the material available at present it is impossible to exclude this possibility, but from what is known of transneuronal degeneration in the mammalian brain it is highly improbable that the very severe cell loss found in the nuclei rotundus and ovoidalis is simply due to the de-afferentation of their cells, especially since adult animals have been used throughout this study. One final consideration which must be mentioned is the possibility that even those nuclei which show no evidence of cellular degeneration after complete removal of the telencephalon do in fact project upon the telencephalon but, like many cells in the mammalian cerebral cortex or the hippocampal pyramids, they are apparently unaffected by axonal section. While it is necessary to point out these possibilities, for the purpose of this discussion we shall assume that the first interpretation is the most probable and shall discuss our findings in this light.

The total thalamic projection upon the telencephalon as determined by the technique of retrograde cell degeneration comprises the three most conspicuous nuclei: nuclei ovoidalis, rotundus and subrotundus; the nuclei of the dorsal group: dorsomedialis anterior, dorsolateralis anterior and posterior and the superficialis parvocellularis, together with the entopeduncular, and the three nuclei posterior to the nucleus rotundus, viz. the postrotundus (including the so-called nucleus postero-intermedialis), the subpretectal nucleus and the posteroventral nucleus. Of these nuclei, the first two and both parts of the dorsolateral nucleus undergo severe cell loss after removal of the telencephalon, but in the remainder the essential cellular change is in the form of shrinkage and pallor. The nuclei which are apparently unaffected by telencephalic removal include, amongst others, such well-defined masses as the so-called lateral geniculate nucleus and habenular nuclear group. The absence of change in the lateral geniculate is particularly deserving of comment in view of the fact that optic nerve fibres have been found to terminate in this nucleus. We have no evidence regarding the projection of this and the other nuclei which are unrelated to the telencephalon.

Although the nuclei which project upon the telencephalon may be divided on the basis of their cellular reaction to telencephalic removal into two main groups, the experiments presented in § II provide another and probably more significant classification because a correlation of the site of the lesion, and the distribution and severity of the resulting thalamic degeneration makes it clear that these nuclei project upon two distinct regions of the telencephalon and do so by way of quite separate efferent pathways. Thus the dorsal group of nuclei have been shown to project through the septo-mesencephalic tract to the dorsomedial margin of the hemisphere while all the other nuclei are related to the palaeostriatum. The connexions of each of these nuclear groups will be considered separately.

The dorsal nuclei

One of the most interesting results of these experiments is the finding that the dorsal nuclei are related not only on topographical grounds but also in their efferent connexions. This group is composed of four nuclei—the nuclei dorsolateralis anterior and posterior, dorsomedialis anterior and superficialis parvocellularis—and these are the only thalamic nuclei which project to the telencephalon outside the palaeostriatum. Although the precise site of termination of the efferent fibres from these nuclei has not been determined there is no doubt that they all project upon the dorsomedial margin of the hemisphere, to either the accessory hyperstriatum or to the adjacent entorhinal cortical area. Furthermore, several experiments have been described which show that these nuclei may degenerate independently of each other, and that there is a topical organization in the projection of this group. It has also been shown that they share a common projection pathway, the septo-mesencephalic tract, and in this respect they differ significantly from the remaining nuclear groups. The probable reason why it has not been possible to determine the site of termination of these nuclei is to be found in the Bodian-stained sections of this material. These sections show that while the septo-mesencephalic tract forms a compact bundle as it passes through the septum and indeed almost as far as the dorsomedial margin of the hemisphere, beyond this its fibres then fan out widely in the anteroposterior and mediolateral directions. It is clear from this that even quite small lesions are likely to interrupt the efferent fibres from more than one nucleus. The significance of the differences in the degree of cellular degeneration which have always been found in the four elements of this group is not at all clear. It does, however, suggest a difference in the pattern of the projection of the nuclei dorsomedialis anterior and superficialis parvocellularis on the one hand and the nuclei dorsolateralis anterior and posterior on the other.

Nuclei rotundus, subrotundus and ovoidalis

It has been possible to determine fairly precisely the projection of these three conspicuous nuclei because the retrograde cell degeneration in these elements is most profound and consequently degenerated areas within them stand out with remarkable clarity. The evidence for the conclusion that all three nuclei project to the palaeostriatum has already been given in some detail in the results, and also that, of the two segments of the palaeostriatum, it is to the palaeostriatum augmentatum rather than to the palaeostriatum primitivum that they send their axons. From a correlation of the site of the damage in the palaeostriatum and the distribution of the degeneration in these nuclei in several experiments it can be stated that these three nuclei project independently of each other, and that within the projection of each nucleus there is a precise topographical organization. In this way it has been demonstrated that the topographical relation of these nuclei to each other is paralleled in the comparable organization of their projection fields. Thus the nucleus rotundus, which in the thalamus is the most lateral of the three and also extends farthest anteriorly, projects to the anterolateral part of the palaeostriatum augmentatum. Similarly, the nucleus ovoidalis which lies most medial, opposite the posterior part of the nucleus rotundus, projects to the posteromedial part of the

palaeostriatum. The precise projection of the nucleus subrotundus is less adequately documented, but it is interesting that all the evidence points to the fact that this nucleus, which is situated between the nuclei rotundus and ovoidalis, projects to an intermediate area in the palaeostriatum. Not only is it clear that these nuclei project to an entirely different part of the telencephalon than the dorsal nuclei, but it has been shown that the course of their efferent fibres through the lateral forebrain bundle is quite separate from that of the dorsal group.

The entopeduncular nucleus and the posterior nuclear group

Little can be added to the conclusions about the projection of these nuclei, which have been already discussed with the results. It is clear that they differ in the pattern of their projection from the other main nuclei in that they undergo only partial degeneration, and that they project together to the same region, as degeneration in one nucleus is always associated with degenerative changes in all the others. They do not degenerate with the dorsal nuclear group, but evidence has been presented that they are always affected when the nuclei rotundus, subrotundus and ovoidalis degenerate, either alone or together. It is not at all clear, however, whether they project in a more or less diffuse way to the palaeostriatum, or whether the degenerative changes seen in these nuclei are the result, not of direct axonal injury, but are in some way secondary to the changes in the nuclei ovoidalis and rotundus. It is nevertheless certain that they do not project diffusely to the whole telencephalon, as will become clear from the fuller discussion of this point below.

The entopeduncular nucleus appears to differ from the nuclei posterior to the nucleus rotundus in at least one respect: in the posterior nuclei degeneration has never been seen to be localized to one part of a given nucleus, but in the entopeduncular nucleus there is evidence of a mediolateral organization comparable to that found in the nucleus rotundus. Thus in one experiment described (OP 5) the medial part of the entopeduncular nucleus has degenerated together with the nucleus ovoidalis, while in another experiment (OP 23) degenerative changes have been found only in the lateral part of the nucleus in association with degeneration in the rotundus.

In this discussion it has been assumed that if retrograde degeneration occurs in a particular nucleus after a lesion in a localized part of the telencephalon, that the projection fibres from that nucleus *terminate* in the damaged area. In the case of the dorsal nuclei this assumption is no doubt justifiable, since the degenerative changes in these nuclei have occurred after relatively superficial lesions and hence presumably at the site of termination of the efferent fibres. On the other hand the validity of this premise may well be questioned for those nuclei which only degenerate after rather deep lesions involving the palaeostriatum. For example, it might be suggested that cellular degeneration in these nuclei is not due so much to the destruction of the termination of their efferents as to damage of the projection fibres as they pass through the palaeostriatum. This possibility cannot be completely excluded as we have no single experiment in which the whole telencephalon, with the exception of the palaeostriatum, has been destroyed, but a number of experiments have been described in which large areas of the telencephalon have been damaged without even minor changes in these thalamic nuclei. Support for this suggestion may be

derived from the older literature in which connexions have been described between these thalamic nuclei and the neostriatum and ektostriatum. These observations appear to be based largely on the study of normal material from which the precise site of termination of fibres or even the direction of conduction cannot usually be determined. In view of these difficulties we shall not compare our findings in relation to specific nuclei with those of previous workers, and judgement on this problem must be suspended pending further study with the more refined silver degeneration techniques after lesions in the thalamus.

In conclusion it may be stated that within the limitations of the method of retrograde cell degeneration the thalamic nuclei appear to project to two distinct parts of the telencephalon—the dorsomedial margin of the hemisphere and the palaeostriatum—and to do so by way of two separate pathways, the septo-mesencephalic tract and the lateral forebrain bundle. The distinctly different structure of these two projection areas strongly suggests that these two groups of nuclei are functionally distinct, and it would not be unlikely if they differed in their afferent connexions.

It would be tempting on the basis of these results to suggest homologies between the various elements of the avian thalamus and those of the reptilian and mammalian brain. For example, there is now evidence that the morphologically similar nucleus rotundus of the lizard and pigeon thalamus both project upon the palaeostriatum; however, in the absence of more precise knowledge of their afferent connexions it would be unwise to conclude that such an homology is in fact established. It is important to emphasize that the avian thalamus as a whole is considerably more developed than that of the reptile and this factor alone should warn against a too-facile drawing of homologies. Similarly, it might be suggested that those nuclei of the avian thalamus which degenerate after removal of the telencephalon are collectively homologous with the dorsal thalamus of the mammalian brain, but further studies, particularly of the afferent connexions, may well show that the avian thalamus is in fact more closely related to the mammalian ventral and/or subthalamus. That homologies with specific nuclei of the mammalian thalamus cannot be drawn is apparent from what is already known of the lateral geniculate nucleus. The absence of retrograde degeneration in this nucleus after complete removal of the telencephalon indicates that it cannot be homologous with that element of the mammalian lateral geniculate body which is derived embryologically from the dorsal thalamus. With regard to the afferent connexions the recent work of Erulkar (1955) has raised another difficulty which must be considered in any discussion of thalamic homologies. Using electro-physiological techniques Erulkar could find no evidence for a thalamic relay of auditory and tactile impulses although responses could be recorded in a localized region of the neostriatum caudale. From these observations he has concluded that the avian thalamus contains no structure homologous with the primary relay nuclei of the mammalian dorsal thalamus. An important problem which these findings raise is the question of the afferent connexions of the cortex, hyperstriatum and neostriatum. It is possible that these areas do receive afferent impulses from the diencephalon or lower levels, and the absence of cellular degeneration in other nuclei of the diencephalon or midbrain (although the latter has not been systematically studied) does not exclude such connexions.

Indeed, the experimental findings of Erulkar (1955) may be taken as evidence of such a connexion between the complex of the nucleus isthmi of the midbrain and the neostriatum caudale, even though in our material these nuclei show no evidence of retrograde cell degeneration after complete removal of the telencephalon. The anatomical basis for the isthmo-striatal relations indicated by Erulkar's work requires further investigation, as does the problem of the source of the afferents to the different telencephalic areas and the elucidation of the organization within the telencephalon.

SUMMARY

1. The total thalamic projection upon the telencephalon and the organization of the projection of the individual nuclei of the thalamus have been investigated in the pigeon (*Columba livia*) using the technique of retrograde cell degeneration.

2. The thalamic nuclei which have been found to project to the telencephalon include the nuclei rotundus, subrotundus, ovoidalis, dorsolateralis anterior and posterior, dorsomedialis anterior, superficialis parvocellularis, postrotundus, posteroventralis, entopeduncularis and the subpretectalis.

3. On the basis of the severity of the cellular reaction to complete removal of the telencephalon these nuclei may be divided into two main groups: those which undergo complete cell loss (e.g. the rotundus) and those in which the essential change is in the form of cell shrinkage and pallor (e.g. the nucleus dorsomedialis anterior).

4. The nuclei which show no change after total ablation of the telencephalon include those of the habenular complex and the so-called lateral geniculate nucleus.

5. The dorsal nuclear group, comprising the nuclei dorsomedialis anterior, dorsolateralis anterior and posterior and the nucleus superficialis parvocellularis, have been found to project upon the dorsomedial margin of the hemisphere (to either the accessory hyperstriatum or the entorhinal cortex or both). The efferent fibres from these nuclei pass in the septo-mesencephalic tract.

6. The central group of nuclei, consisting of the three most conspicuous thalamic nuclei, viz. nuclei rotundus, subrotundus and ovoidalis, are related to the palaeostriatum augmentatum. These nuclei project independently of each other and within the projection of each of these nuclei there is a precise topical organization. The efferent fibres from these nuclei pass through the lateral forebrain-bundle.

7. It has not been possible to define the precise mode of projection of the entopeduncular nucleus or of the nuclei of the posterior group (nuclei posteroventralis, postrotundus and subpretectalis), but it appears that they either project upon the palaeostriatum augmentatum or alternatively the degeneration seen in these nuclei after lesions of the palaeostriatum is secondary to that found in the three central nuclei.

8. It does not appear possible, solely on the basis of the telencephalic projection, to homologize individual elements of avian thalamus with specific nuclei or nuclear groups in either the reptilian or mammalian thalamus.

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ABBREVIATIONS

AHS	Accessory hyperstriatum
AS	Archistriatum
C	Cerebellum
DLC	Dorsolateral cortex
EA	Entorhinal area
EM	Nucleus ectomamillaris
EN	Entopeduncular nucleus
H	Hippocampal cortex
HS	Hyperstriatum
LFB	Lateral forebrain bundle
LGN	Lateral geniculate nucleus
LHN	Lateral habenular nucleus
MFB	Medial forebrain bundle
MHN	Medial habenular nucleus
MT	Marginal tract
NDLA	Nucleus dorsolateralis anterior
NDLP	Nucleus dorsolateralis posterior
NDM	Nucleus dorsomedialis anterior
NO	Nucleus ovoidalis
NR	Nucleus rotundus
NS	Neostriatum
NSC	Neostriatum caudale
NSF	Neostriatum frontale
NSI	Neostriatum intermediale
NSP	Nucleus superficialis parvocellular'
NSPI	Nucleus spiriformis
NSR	Nucleus subrotundus
OB	Olfactory bulb
OC	Optic chiasma
OT	Optic tract
OTC	Optic tectum
PC	Pyriform cortex
PSA	Palaeostriatum augmentatum
PSP	Palaeostriatum primitivum
S	Septum
SMT	Septo-mesencephalic tract
TG	Nucleus subpretectalis
VP	Nucleus ventralis posterior

EXPLANATION OF PLATES

All the figures are photomicrographs of thionine stained sections.

Plate 1

Fig. 1. A low-power photomicrograph of a transverse section through the telencephalon just rostral to the septum to show the extent of the lesion in the right hemisphere in experiment OP 20. ×11.

Fig. 2. Photomicrograph of a section through the middle of the thalamus to show the severity and extent of the degeneration in the nuclei of the central and dorsal groups in experiment OP 20. ×24.

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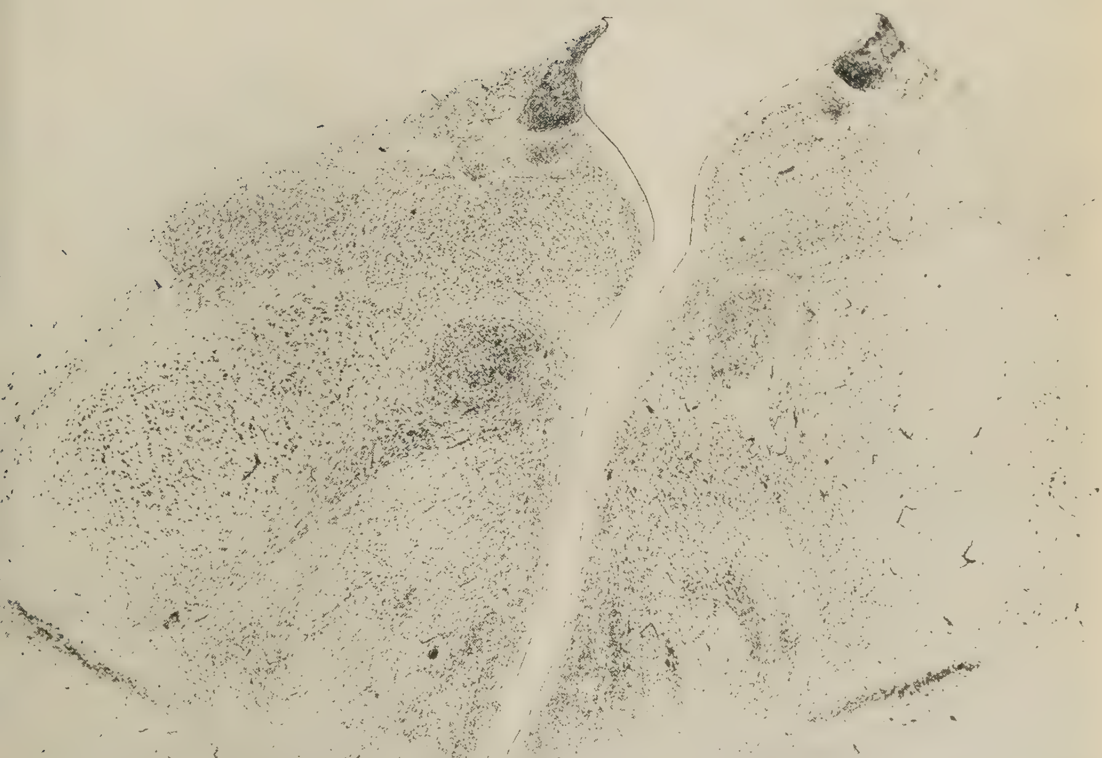
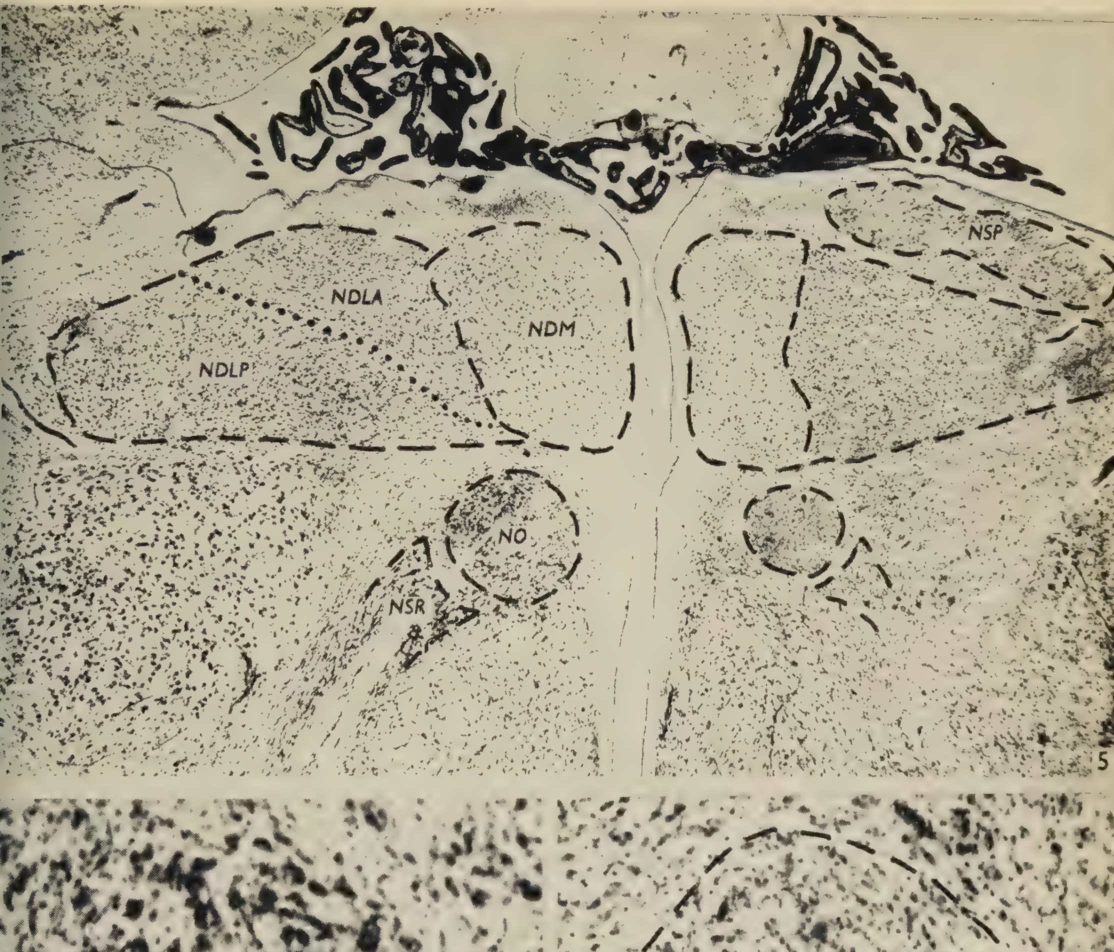


2

POWELL AND COWAN—THALAMIC PROJECTION UPON TELENCEPHALON IN THE PIGEON

(*Facing p.* 108)

POWELL AND COWAN—THALAMIC PROJECTION UPON TELENCEPHALON IN THE PIGEON



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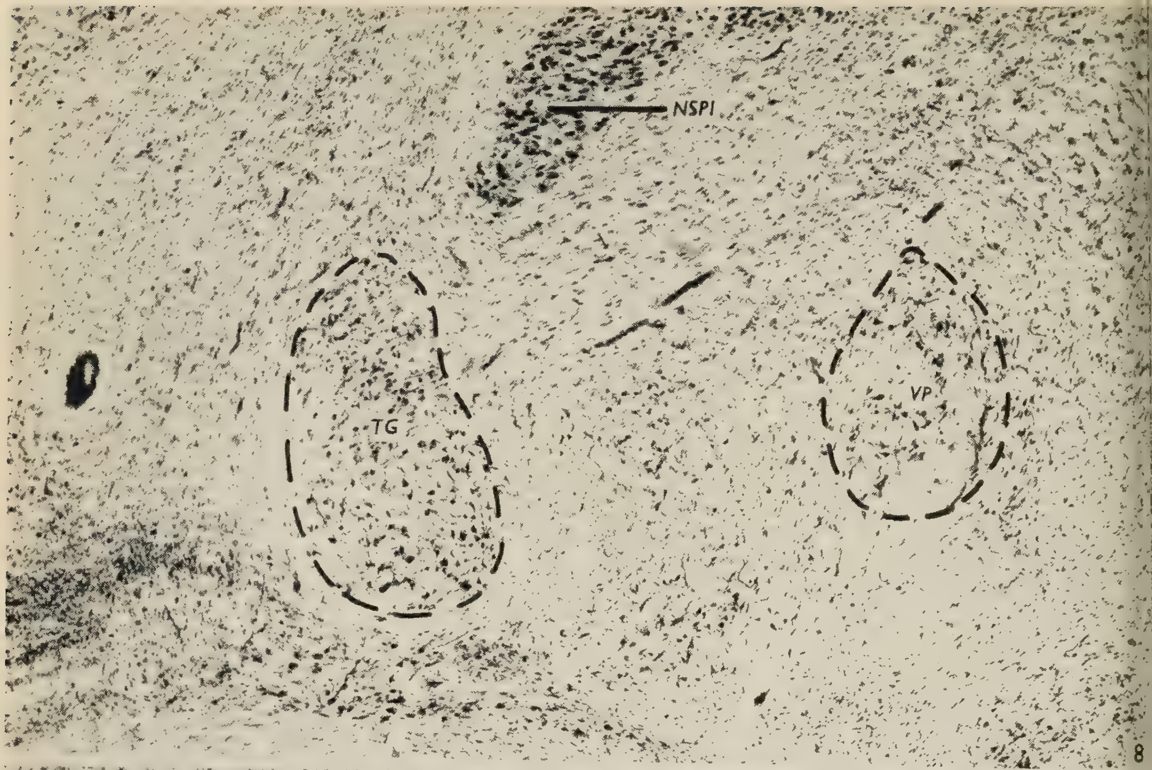

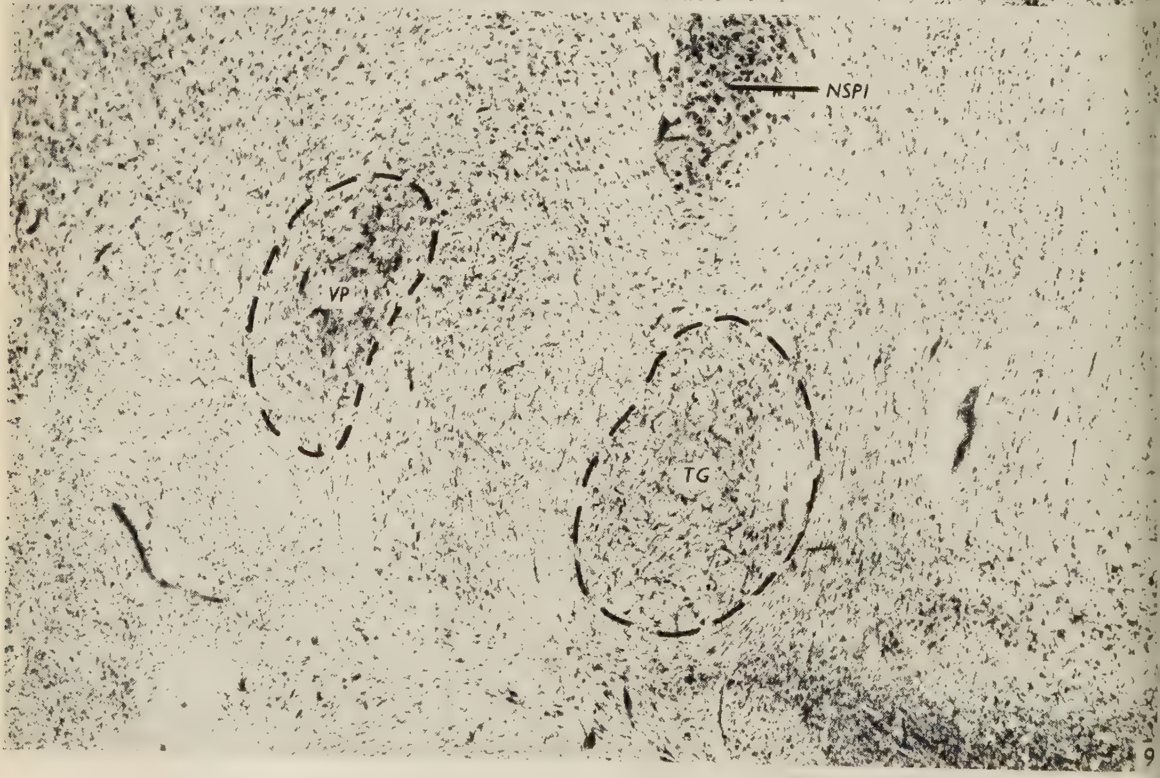

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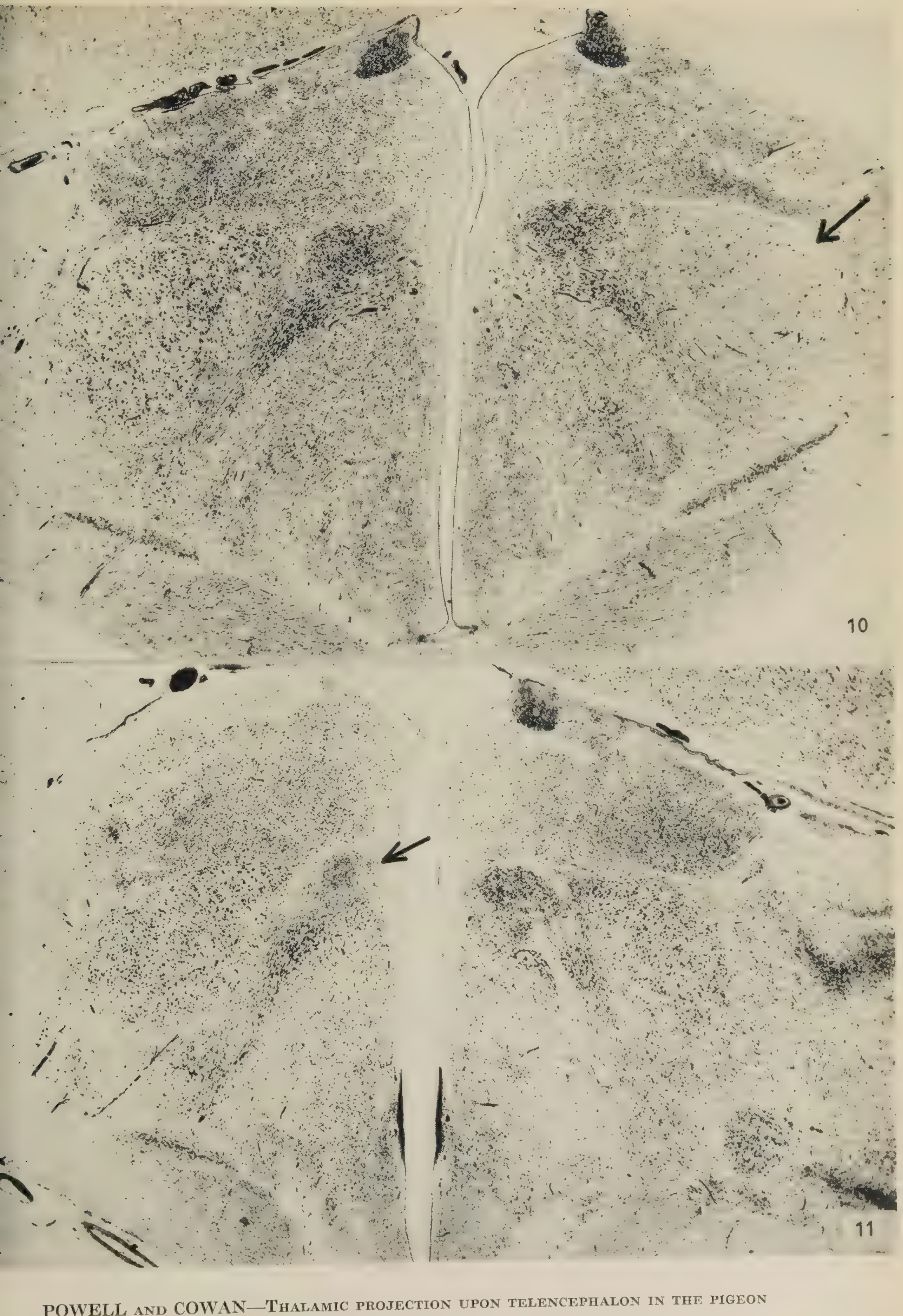

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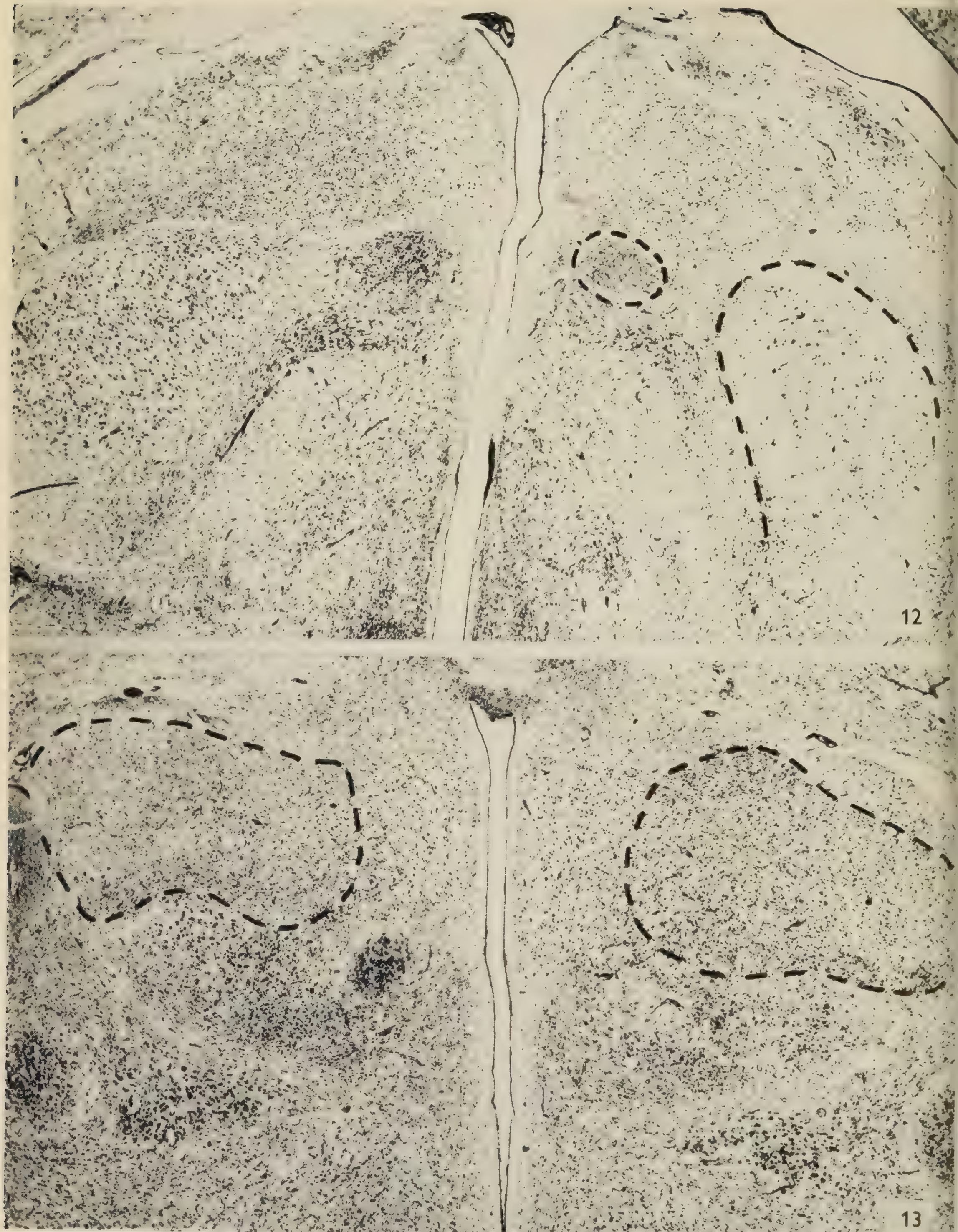

POWELL AND COWAN—THALAMIC PROJECTION UPON TELENCEPHALON IN THE PIGEON

PLATE 2

Figs. 3, 4. Photomicrograph at higher magnification to show the severity of the degenerative changes in the central group of nuclei: nucleus rotundus, ovoidalis and subrotundus compared with the normal side. In Fig. 3 the normal appearance of these nuclei on the unoperated side is shown; the degenerated nuclei of the operated side are outlined in Fig. 4. $\times 42$.

PLATE 3

Fig. 5. A low-power photomicrograph of the dorsal nuclei in experiment OP 30 to show the extent of the degeneration in the dorsolateral and dorsomedial nuclei. The dotted line marks the boundary between the nuclei dorsolateralis anterior and posterior on the normal side. The degenerated nuclei ovoidalis and subrotundus are also shown. $\times 24$.

Figs. 6, 7. High-power photomicrograph of the normal nucleus ovoidalis (Fig. 6) and the degenerated nucleus of the opposite side (Fig. 7) in experiment OP 20. $\times 154$.

PLATE 4

Figs. 8, 9. The nuclei ventralis posterior and subpretectalis on the normal (Fig. 8) and operated (Fig. 9) sides in experiment OP 20. Note the absence of changes in the adjacent nucleus spiriformis. $\times 42$.

PLATE 5

Fig. 10. The thalamus of experiment OP 23 to show the severe retrograde cell degeneration in nucleus rotundus (indicated by arrow). Note the absence of degeneration in nucleus ovoidalis. $\times 24$.

Fig. 11. The thalamus of experiment OP 31 to show the degeneration in the nucleus ovoidalis of the left side (indicated by arrow). Note the preservation of the nuclei rotundus and subrotundus. $\times 24$.

PLATE 6

Fig. 12. Photomicrograph of a transverse section of the thalamus of experiment OP 21 to show the virtual preservation of the nucleus subrotundus in contrast to the severe degeneration of the adjacent nuclei rotundus and ovoidalis (outlined by broken lines). $\times 24$.

Fig. 13. Photomicrograph of a horizontal section of the thalamus of experiment OP 63 to show the marked degree of retrograde cell degeneration in the nuclei dorsolateralis anterior and posterior of the left side. The nucleus dorsomedialis anterior shows little change. $\times 24$.

THE EFFECT OF AGE ON THE ARRANGEMENT OF FIBRES IN THE BONE MATRIX OF THE FEMUR OF THE DOMESTIC FOWL

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INTRODUCTION

In previous papers (Pratt, 1957, 1959) accounts were given of the fibre structure of the various kinds of bone present in the prenatal and postnatal femur of the rat. The present paper extends these studies to the domestic fowl, and again special attention has been given to the situation of the various kinds of bone in relation to the growth of the bone as a whole. The arrangement of collagen fibre bundles in the matrix of avian bone was described by von Ebner (1875) and later by Weidenreich (1923), but both of these accounts were concerned with the classification of bony tissue according to its fibrous structure and did not consider the bone as a whole. There is also the description of avian bone by Maj (1938), as seen with polarized light, but he did not describe the fibre pattern in any detail. There do not appear to be any previous accounts of the structural changes associated with the maturation of avian long bones apart from that of Fell (1925) which covers the embryonic period, and the brief account of Policard (1941) which does not extend beyond the third month of postnatal life. It was considered, therefore, a necessary preliminary to include a brief account of the maturation of the fowl's femur.

MATERIALS AND METHOD

The material used included embryos of 9, 11, 13 and 17 days, postnatal cockerels aged 1, 8, 15, 22, 29, 43, 57, 71, 85, 120, 155, 190, 246 and 379 days, and two laying hens, all of which were from the same Rhode Island Red stock.

The embryos were fixed in Bouin's fluid and the hind limb was removed subsequently on each side. In the case of the postnatal birds the femur was dissected out on each side immediately after death and fixed in 5% formal saline. The bones were decalcified with the disodium salt of ethylene-diamine-tetracetic acid, and in each case the specimen from one side was cut longitudinally and that from the other side was cut transversely. Sections thus obtained at 4 μ were stained with haematoxylin and eosin, iron haematoxylin, by a modification of Long's silver impregnation which showed both reticular and collagenous fibres, and with Weigert's elastin stain.

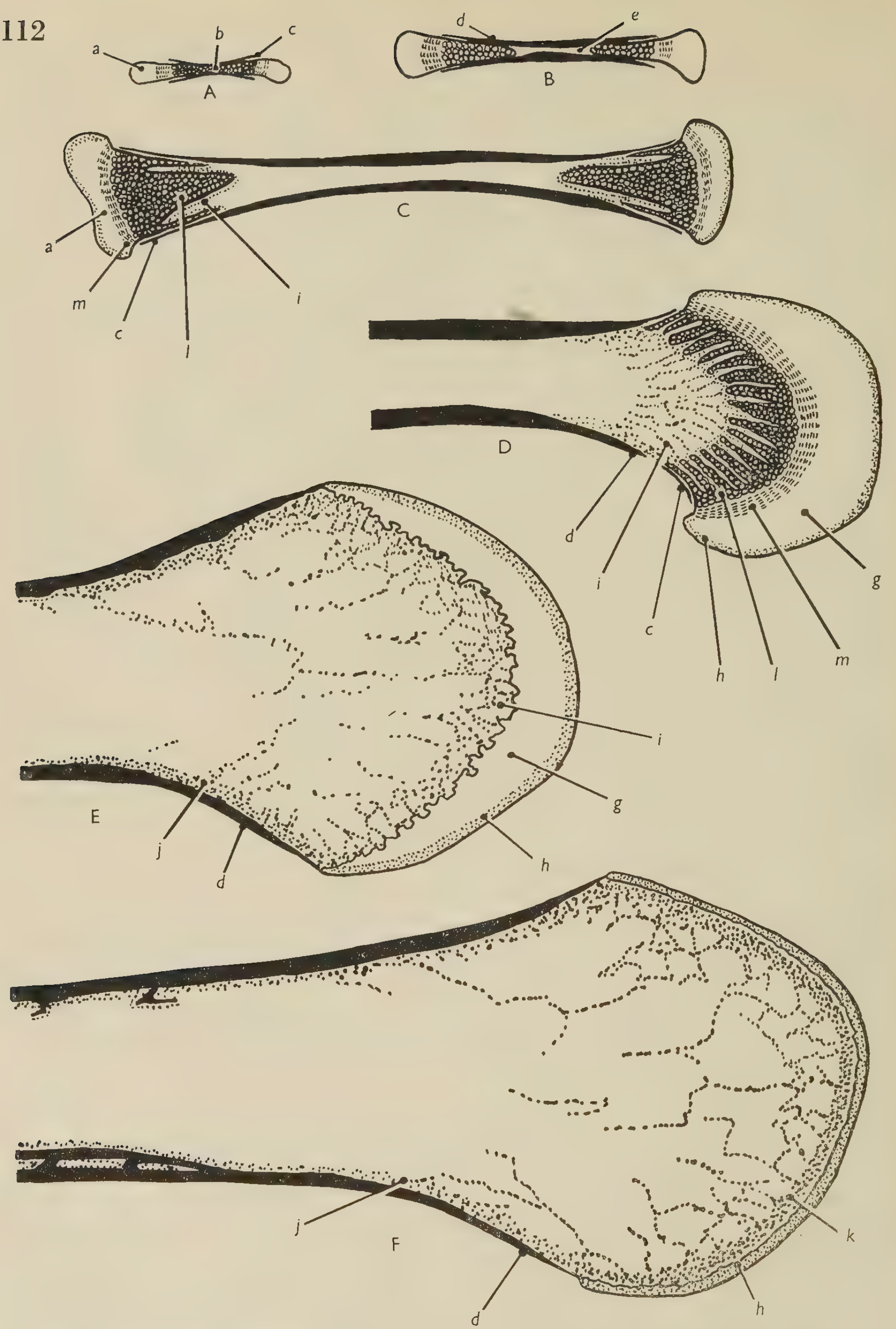
OBSERVATIONS

Text-fig. 1 outlines the main features of the development and maturation of the fowl's femur, and it will be seen that there are several differences when these are compared with the better known patterns of development and maturation of mammalian long bones. In the earliest stage examined (Text-fig. 1A), the arrangement

of the constituent cells of the cartilage model allows one to distinguish between the central diaphysial cartilage and the two peripheral epiphysial cartilages. The central portion of the diaphysial cartilage has undergone hypertrophic changes and is surrounded by a sleeve of perichondrial bone. This cartilage is then rapidly invaded by subperiosteal tissues, and a primary medullary cavity is formed. Meanwhile a shaft of periosteal bone has been laid down around the diaphysial cartilage (Text-fig. 1B), and by the time of hatching the shaft consists solely of periosteal bone. This is closed at both extremities by the remains of the diaphysial cartilage, each of which now forms a growth cartilage consisting of the juxta-medullary hypertrophic zone and the juxta-epiphysial proliferative zone (Text-fig. 1C). Trabeculae of endochondral bone appear for the first time in the metaphysis of the postnatal bone. The walls of the latter, now and in all later stages, are formed largely of periosteal bone (Text-fig. 1D, E, F). This bone is separated at first by perichondrial bone from the underlying endochondral bone. Later, if external metaphysial remodelling occurs, the periosteal bone is separated by a cement line from the underlying endochondral bone and endosteal bone. The structure of the avian growth and epiphysial cartilages has been described by Haines (1942) and Wolbach & Hegsted (1952). The medullary aspect of the growth cartilage (the hypertrophic zone) is continuously, but unevenly, invaded by marrow tissues, which gives a serrated appearance to the cartilage. The epiphysial cartilage does not undergo endochondral ossification in the way it does in mammals, but persists for a considerable time as a wide basophilic hyaline zone and a narrow outer eosinophilic articular zone (Text-fig. 1D, E). The cells of the growth cartilage become exhausted with maturity and this structure has disappeared at both extremities by 155 days (Text-fig. 1E). The invading marrow tissues then enter the hyaline zone of the epiphysial cartilage, where individual groups of chondrocytes have hypertrophed and in this way endochondral osteogenesis slowly spreads through the hyaline zone, so that by 190 days only the articular zone persists, whose structure at this stage is described by Whiston (1940). At this point a terminal plate of bone is formed which lines the deep surface of the articular cartilage (Text-fig. 1F). Though not illustrated in the text-figure, there is an extensive deposition of endosteal bone throughout, and almost completely filling, the medullary cavity of the long bones of female birds during the egg-laying cycle. The process has been described in the domestic fowl by Bloom, Domm, Nalbandov & Bloom (1958).

(i) *Perichondrial bone*

The perichondrial bone (the 'primary diaphysial lamella' of Johnston, 1958) is laid down under the perichondrium and directly upon the surface of the diaphysial cartilage. This bone appears to be devoid of lacunae and is about $10\,\mu$ in thickness. Its fibrous structure is distinctive and consists of short fine fibres, which are directed radially and densely packed (Pl. 2, fig. 4). In the 9-day-old embryo this bone forms a continuous thin sleeve, which is co-extensive with the zone of hypertrophic cartilage. Further perichondrial bone is being formed at the junction of the hypertrophic and proliferative zones, about which point the extremities of the diaphysial cartilage are increasing in diameter as well as elongating, and as a result the perichondrial bone has an hour-glass shape. An erosion of the central portion of the



Text-fig. 1. Longitudinal sections of the fowl's femur showing the changes which occur during development and maturation. A, 9-day-old embryo; B, 13-day-old embryo; C, 1-day-old cockerel; D, 29-day-old cockerel; E, 155-day-old cockerel; F, 190-day-old cockerel. (In D, E and F the distal extremity is shown.) ×4. *a*, epiphysial cartilage; *b*, diaphysial cartilage; *c*, perichondrial bone; *d*, periosteal bone; *e*, primary medullary cavity; *g*, hyaline zone (of epiphysial cartilage); *h*, articular zone (of epiphysial cartilage); *i*, endochondral bone; *j*, endosteal bone; *k*, terminal plate of bone; *l*, hypertrophic zone (of growth cartilage); *m*, proliferative zone (of growth cartilage).

perichondrial bone has occurred by the 13th day of embryonic life, when, at the same time the central part of the diaphysial cartilage is completely replaced by a marrow cavity. With subsequent medullary erosion the perichondrial bone comes to line the marrow cavity, but in the later embryonic stages a narrow band of cartilage remains unabsorbed, and lies immediately adjacent to the perichondrial bone. By 17 days the perichondrial bone is confined to the metaphysis.

Perichondrial bone formation continues in postnatal bones, where it occurs between the perichondrium and the hypertrophic zone of the growth cartilage. With subsequent growth this bone forms part of the wall of the metaphysis unless there is external remodelling of the latter when the perichondrial bone forms a rather ill defined 'perichondrial ring of the ossification groove' (Text-fig. 1D) which is co-extensive with the hypertrophic zone of the growth cartilage. The fibres of the postnatal perichondrial bone are irregularly arranged and it is difficult to distinguish between this bone and the periosteal bone which becomes deposited on its outer surface.

(ii) *The periosteal bone*

The fibrous perichondrium surrounding the bones of the early embryo is, by convention, referred to as the periosteum in the later embryos, as perichondrial bone has appeared, and any further bone that may be deposited beneath this fibrous sheath is called periosteal bone.

(A) *Embryonic woven bone.* This bone exists in two forms, which will be referred to as early and late periosteal bone. The early periosteal bone lies about the middle of the shaft and consists of irregular trabeculae, containing numerous large round closely packed lacunae. The fibres of its matrix are finely bundled and form an irregular network which blends both with the fibres of the periosteum and with the fibres of the perichondrial bone (Pl. 2, fig. 4). On the other hand, the late periosteal bone (*Fasenknochen* of Weidenreich, 1923), which first is confined to the extremities of the shaft, consists of elongated needle-like trabeculae, these being longitudinally directed and giving an imbricated appearance. The matrix of this bone contains coarse longitudinally directed fibre bundles (Pl. 2, fig. 5) which form the 'core' of the trabeculae where they lie in a fine network of fibres similar to that found in the early periosteal bone.

(B) *Neonatal sheath.* This matrix appears to have escaped recognition in the past. It is not apparent in haematoxylin and eosin preparations, but in silver-stained sections it appears as a densely fibred band. It forms at the time of hatching, when a layer (about 5μ in thickness) of densely packed longitudinally running fibre bundles is deposited about the whole of the circumference of the bone. The appearances at 8 days confirm the fact that it forms a compete sheath to the bone, though by this time further deposition of woven bone has occurred and the neonatal sheath no longer lies on the external surface of the bone (Pl. 1, fig. 1; Pl. 2, fig. 5). The progressive enlargement of the medullary cavity results in the disappearances of the sheath by 29 days. The persistence of the neonatal sheath within the shaft during the first 4 weeks of postnatal life makes it possible to use the sheath as a marker for indicating growth in width during this period (Text-fig. 2). It will be seen that during this period periosteal deposition and medullary remodelling are

eccentric, and a considerable portion of the bone is unchanged for at least the first 3 weeks.

(C) *Postnatal woven bone.* The bone matrix formed after the appearance of the neonatal sheath is similar to the late embryonic periosteal bone, but in the former the incorporation of extra-osseous fibres plays an increasingly important part. The postnatal fibrous periosteum contains fine reticular fibres, wavy collagen fibres and linear elastic fibres, all of which are tightly packed and run longitudinally. Immediately adjacent to the deep surface of the fibrous periosteum lie osteoblasts, in varying stages of differentiation, and situated within a dense network of fibres. Some of these latter fibres are collected into linear bundles of wavy fibres, which at one extremity appear to be continuous with the fibrous periosteum and at the other extremity pass into the bone where they can often be traced to the medullary surface. These osseous fibre bundles, which were observed by Gegenbaur (1867), increase in number with age and run parallel to each other in either half of the bone, always being directed outwards towards the nearest extremity, which means that at either extremity they will be running almost longitudinally but will be radially directed about the middle of the bone. These osseous fibre bundles appear to act as a scaffolding for the formation of the trabeculae of more finely fibred bone. This latter bone matrix is finely and irregularly fibred similar to the early embryonic periosteal bone, and contains numerous spherical lacunae (Pl. 2, fig. 5). Vascular canals form by the bridging of adjacent trabeculae (Pl. 1, fig. 2), and these channels become further reduced in diameter by the deposition within them of bone whose matrix is characterized by the more orderly arrangement of the fine fibre bundles which run, closely packed, in the long axis of the vascular channel, as described by von Ebner (1875) (Pl. 1, fig. 2; Pl. 2, fig. 10). These perivascular structures correspond to the primary osteones described in reptiles by Gross (1934).

There is an incorporation of tendon fibres into the bone in certain situations. These fibres are similar to the osseous fibre bundles but are thicker (Pl. 3, fig. 14). In some instances the tendon fibres may cross the osseous fibre bundles and the former are often to be seen transversing vascular spaces and then re-entering bone substance.

The vascular spaces found in the wall of the shaft, whose formation has already been described, in some instances acquire a lining which appears to be devoid of fibres (Pl. 3, fig. 11). This hyaline lining, which does not appear to have been described in the past, varies from 2 to 7 μ in thickness and contains lacunae occupied by elongated cells. These structures appear to be confined to relatively 'old' parts of the bone. For example, such linings are found even in early stages in bone adjacent to a periosteal surface which has been static for a time due to an eccentric mode of growth. After 85 days, when there is little medullary erosion of the shaft,

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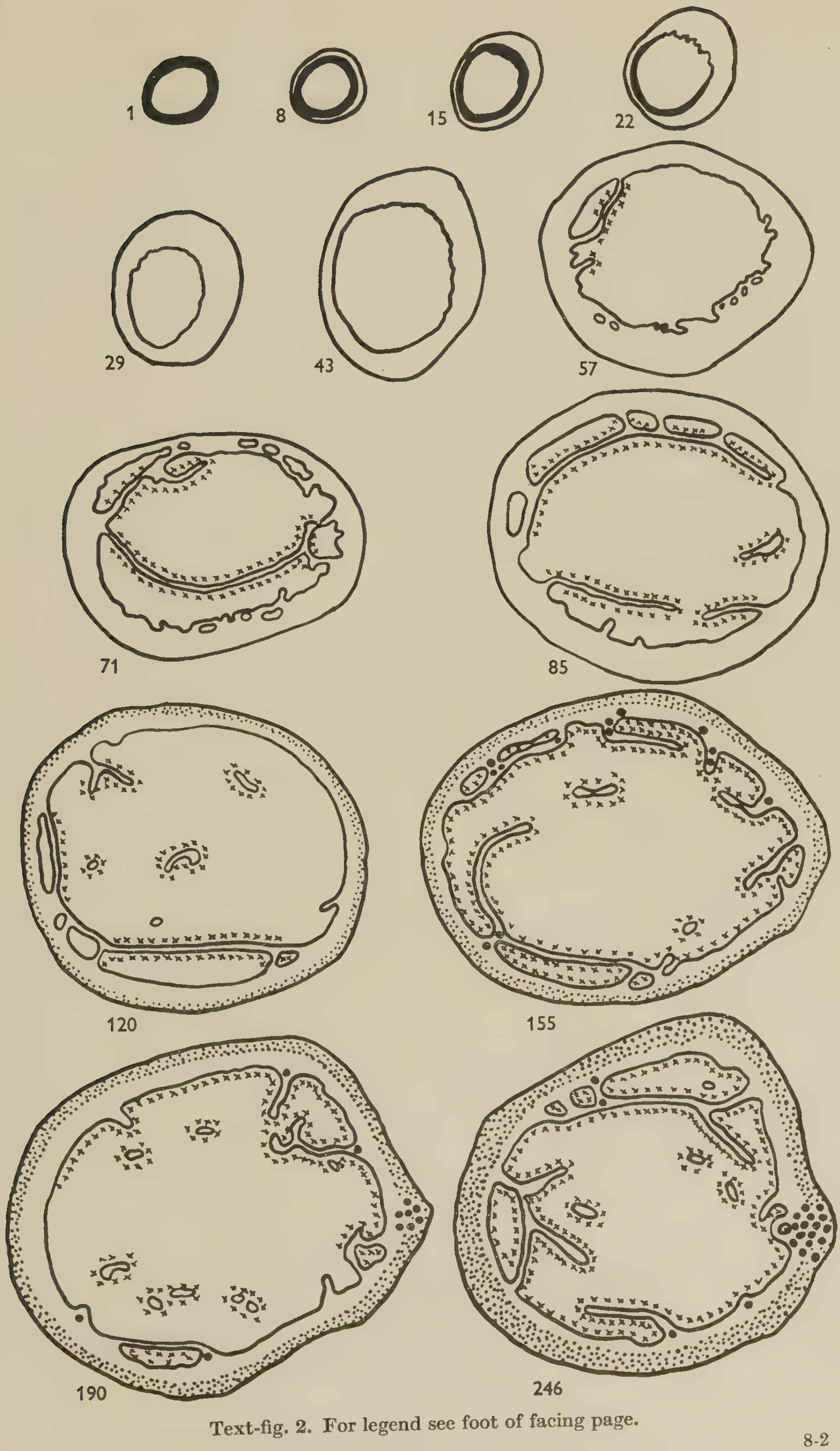
Text-fig. 2. Transverse sections of the diaphysis of the femur from cockerels aged 1–246 days. Sites of endosteal ossification are shown with crosses; it should be noted that this process occurs in the first instance upon the intramedullary trabeculae, and only later occurs throughout the medullary cavity. The bone formed during embryonic life is identified by the presence of the neonatal sheath, which limits this bone, which is shown as solid in the first four stages illustrated. The lictor-bundle bone, which also contains elastic fibres, which is laid down after 85 days, is shown by stippling. Secondary osteones are shown as solid circles ($\times 5\frac{1}{2}$).



Text-fig. 2. For legend see foot of facing page.

the remaining woven cancellous bone persists indefinitely, and consequently most of vascular spaces within this bone have hyaline linings (Pl. 1, fig. 3).

(D) *Lictor-bundle bone.* At 120 days and in all later stages (Text-fig. 2) the newly formed matrix of the periosteal bone has a homogeneous appearance (Pl. 1, fig. 3), as it consists almost entirely of longitudinally arranged and densely packed fibre bundles (Pl. 2, figs. 6, 7). These fibre bundles have a similar diameter to the perivascular fibre bundles seen in the earlier stages. Their wavy appearance and their linear arrangement was observed by Weidenreich (1930), who called this type of bone lictor-bundle bone. Though usually forming fine fibre bundles they do collect into coarser bundles in some situations. The lacunae are elongated and fewer than in the woven bone. Occasional circumferential bundles of fibres are to be seen, which result in a type of lamellar bone.

It would seem that this late periosteal bone results in part from an incorporation of the deeper layers of the fibrous periosteum. Elastic fibres are found within the matrix of the periosteal bone in all the specimens examined at 120 days and later. These fibres are not seen in the bone matrix in earlier stages but are present in the fibrous periosteum. When in the bone matrix they appear as incomplete concentric sheaths of longitudinally running fibres (Pl. 2, figs. 8, 9). Elastic fibres have been described previously in avian bone (Weidenreich, 1930), but the significance of their incorporation into bone has not been commented upon.

(E) *Secondary osteones.* Secondary osteones, which are perivascular deposits of bone separated from the surrounding bone by reversal lines, are occasionally found within the cancellous woven bone which persists in the diaphysis of older birds (Text-fig. 2; Pl. 1, fig. 3). The presence of these structures suggests that earlier there has been localized resorption of bone, followed by repair. It should be emphasized that secondary osteones are rare and are easily overlooked. Two varieties exist, which are distinguishable structurally, namely those found in areas where tendon fibres pass into the diaphysis, and those found in that part of the diaphysis which is adjacent to the medullary cavity.

In those areas where coarse bundles of tendon fibres pass into the bone, and form a large proportion of the fibrous matrix, secondary osteones are frequently seen after the age of 190 days in both sexes. The lacunae of these osteones are irregularly shaped and have no apparent pattern of distribution. The tightly packed fibre bundles have a diameter similar to that of the bundles of lictor-bundle bone, and run in the same plane as the long axis of the osteone (Pl. 3, fig. 14).

The other type of secondary osteone is seen at 155 days and in older stages in situations adjacent to the medullary surface of the shaft. In these, the lacunae are elongated and arranged circumferentially in a regular manner. The fibres are finely bundled and usually a lamellar arrangement is present, though this may not always be well defined (Pl. 3, fig. 13).

(iii) *Intramedullary bone*

(A) *Embryonic endochondral woven bone.* There is no evidence of intramedullary bone formation until immediately before hatching, when the hypertrophic cartilage, as a consequence of peripheral erosion, forms a large central core (Text-fig. 1C), and the endochondral bone present forms a discontinuous layer on the surface of this

core. It has already been pointed out that a layer of hypertrophic cartilage persists on the internal surface of the perichondrial bone, and endochondral bone is also deposited on the medullary surface of this cartilage. Thus a layer of cartilage comes to be between perichondrial bone on the outside and endochondral bone internally. The fibrous structure of the endochondral bone closely resembles that of the early periosteal bone and consists of an irregular network of fine fibre bundles.

(B) *Postnatal endochondral and endosteal metaphysial woven bone.* Endochondral bone formation continues until both the growth cartilage and the hyaline zone of the epiphysial cartilage are completely exhausted, which has occurred at 190 days (Text-fig. 1). This bone differs from the bone formed in similar situations in the embryo in that it is more extensive and contains numerous rounded osteocytes (Pl. 3, fig. 12). This bone is confined to the metaphysis, due, presumably, to the extensive intramedullary resorption of bone in the region of the junction of the diaphysis and metaphysis, where there are large numbers of osteoclasts. Osteoclasts are also found in large numbers under certain parts of the metaphysial periosteum, as described by Koelliker (1873) when the peripheral trabeculae of endochondral bone are removed during the process of external remodelling of the metaphysis. Elsewhere osteogenesis continues especially amongst those remaining peripheral trabeculae which form the wall of the metaphysis. Subsequently, periosteal bone is deposited on these and a cement line intervenes. This 'endosteal' activity results in the consolidation of the trabeculae of endochondral bone. Cement lines are often also present in this metaphysial spongiosa and serve to distinguish the endosteal from the endochondral bone as the fibrous structure of the two types of bone is identical at this stage.

(C) *Endosteal lictor-bundle and lamellar bone.* The osteoclastic erosion of the internal surface of the diaphysial wall associated with expansion of the marrow cavity occurs at first at all points, later (at 57 days) this process becomes confined to certain situations where, once having penetrated the wall of the shaft, the invading tissues extend circumferentially. This has the effect, by 71 days, of isolating extensive sheets of bone which were originally formed under the periosteum but now lie within and extend throughout the medullary cavity (Text-fig. 2). These trabeculae may subsequently be remodelled to some extent, but are always to be seen in the later postnatal stages. Lictor-bundle bone is deposited on these early intermedullary trabeculae. This bone has sparse and elongated lacunae and is separated from the underlying woven bone by a continuous cement line. The osteoblastic activity associated with the endosteal osteogenesis of the shaft appears to be continuous with the endosteal bone formation occurring in the metaphysis where, by this time, lictor-bundle bone is being formed and woven bone has ceased to be formed.

The endosteal bone, which is formed after 71 days, has a lamellar fibrous structure (Pl. 3, fig. 15) and after 120 days is found lining the whole of the medullary cavity (Text-fig. 2). In the shaft it forms a wide almost avascular layer separated by a smooth cement (resting) line from the bone of periosteal origin (Pl. 1, fig. 3).

(D) *Endosteal bone, associated with egg laying.* In the two hens examined, one of which was in lay and the other was out of lay and moulting, a type of bone, somewhat similar to the early embryonic woven bone, is found forming trabeculae throughout the medullary cavity. Elsewhere this bone is superimposed upon the endosteal

lamellar bone, where the junctions of the two types of bone are sharply demarcated although there are no cement lines, which suggests that there is a continuity of the osteogenic processes. The matrix of this bone is more deeply basophilic than the matrix from any other part of the bone. This bone, associated with egg laying, is irregularly and finely fibred (Pl. 3, fig. 16), and furthermore the fibre density is variable so that some areas appear almost hyaline. It contains numerous irregularly shaped lacunae.

DISCUSSION

Periosteal osteogenesis in relation to growth in length

Experimental data on the growth in length of avian long bones does not seem to be available. There is, however, indirect evidence based on the measurements of large numbers of bones obtained from birds of known ages. Latimer (1927) showed that the femur in White Leghorn cockerels grew in length at an even rate until about 145 days when the rate decreased, so that by about 182 days growth in length had ceased. Buckner, Insko, Harns, Wachs & Wachs (1950), who used New Hampshire cockerels, showed that the femur reached its maximum length by about 119 days. The evidence obtained from the present material is in close agreement with these earlier results, for the disappearance of the growth cartilage by 155 days must mean that rapid elongation will have ceased between 120 and 155 days, but the persistence of the hyaline part of the epiphysial cartilage until 190 days may allow for slight further elongation.

The cessation in the formation of woven bone, and the appearance of lictor-bundle bone, occurs between 85 and 120 days. This period is probably somewhat before the actual cessation of elongation, but a slowing of growth will almost certainly have occurred. It would seem that lictor-bundle bone formation only occurs in any amount when growth in length is very slow or has ceased, and furthermore this bone continues to be laid down for a considerable time after elongation has ceased, which means that the structure of the shaft continues to change after the apparent maturation of the bone. It will be recalled that the elastic fibres are found in the bone amongst the fibres of the lictor-bundle bone, and it is suggested that their presence indicates that there is an incorporation of the fibrous periosteum into the bone as the bone ceases to elongate.

Rapid elongation of the bone would appear to be necessary for the incorporation of the extra-osseous fibres, into its matrix, as is suggested by the normal growth pattern and by the fact that during prolonged starvation, when growth is severely retarded, there are no extra-osseous fibres incorporated into the bone and the fibres found in this bone differ from those in the bones of adults (Pratt & McCance, 1960). These effects upon the fibrous structure of the periosteal bone by the slowing down of the rate of elongation may well explain the formation of the neonatal sheath, when for a short period of time the incorporation of extra-osseous fibres is disturbed.

Perivascular bone

The vascular spaces within the walls of the embryonic diaphysis have no distinct fibrous lining. However, after hatching these and similar vascular spaces become reduced in their diameter by the deposition within them of longitudinally running

and densely packed fibre bundles. The further reduction in the diameter of some of these primary osteones, especially the older ones, by a lining of hyaline matrix, is difficult to understand, and does not seem to have been observed in bone before. It is unlikely that they represent a preosseous zone as they are not found in areas where primary osteones are forming. It is likely, on the other hand, that they are in the nature of a wide resting line similar to those seen in severely dwarfed bones (Pratt & McCance, 1960).

Secondary osteones, which are perivascular bony structures separated from the surrounding bone matrix by a narrow crenated cement line, indicating that they have formed as a result of localized resorption of perivascular bone which has been followed by repair, are rarely seen in the material examined. The two types of fibrous structure seen within these secondary osteones require comment. Those osteones with a lamellar structure, which have also been described by Amprino & Godina (1947) in the phalanx of the ostrich, represent a continuation of endosteal osteogenesis along the perivascular spaces leading from the marrow into the cortex. While those osteones with dense coarse fibres, which appear to be similar to the osteones described by Amprino & Godina (1947) in the metatarsal of the stork, and by Amprino (1948) in the ossifying tendon of a bird, represent a continuation of the periosteal osteogenic process. Thus it would seem that the appearances of secondary osteones depends whether they are repaired by subperiosteal or medullary osteoblasts.

The perivascular structures seen in severely undernourished immature cockerels (Pratt & McCance, 1960) are not secondary osteones, even though they are limited by a cement line. These cement lines are smooth, very wide and are not sharply demarcated, that is they are resting lines, which indicate the cessation of deposition followed by the recommencement of perivascular osteogenesis.

A more extensive development of secondary osteones can occur as shown by Bloom *et al.* (1958) who described cortical erosions, resulting from the enlargement of the vascular channels, in calcium-deficient laying pullets. With their recovery there was a subsequent deposition of bone within the erosions resulting in extensive secondary osteone formation. Similar cortical erosions and their subsequent repair have been described in ducks and waders by Meister (1951), who ascribed these changes to moulting, and recently Zahnd (1954) described similar findings in moulting cocks and hens. However, Urist & Deutsch (1960), when examining fowl bones, found cortical erosion only in hens, and then only after prolonged egg production, and noted further erosion occurred with moulting; but these workers claimed that there was never any repair of these erosions. Though the repair of cortical erosions may fail, this was not the case in the birds examined here. Possibly erosions may have different causes or perhaps capacity for repair can be lost. While secondary osteones are found in the fowl long bone, they are not usually very much in evidence, and it is difficult to assess the significance of their more extensive occurrence in the bones of other birds as described by Foote (1916), Demeter & Mátyás (1928), Maj (1938), Amprino (1952), Amprino & Godina (1944) and Enlow & Brown (1957). Amprino & Godina (1947) pointed out that secondary osteones in birds were found in much greater numbers in the distal limb bones than in the femur.

SUMMARY

1. A brief account of the development and maturation of the fowl's femur is given.

2. The arrangement of fibre bundles in the bone matrix is described as seen following silver impregnation. Several distinctive types of bone are present.

3. Perichondrial bone contains short radially directed fibres in embryonic stages, but is more irregularly fibred in later stages.

4. Finely fibred woven bone is formed under the periosteum in embryonic and early postnatal stages.

5. Extra-osseous fibres are incorporated into bone and result in the imbricated appearance of the trabeculae.

6. A transient neonatal sheath is formed and consists of densely packed longitudinal fibres.

7. The reduction in the diameters of the vascular spaces occurs by means of the deposition of parallel and densely fibred bone. After some time these spaces are further reduced in diameter by a lining of fibreless matrix.

8. The late periosteal bone contains closely packed, longitudinally running lictor bundles, and after 120 days contains large numbers of elastic fibres.

9. Occasional secondary osteones are found in mature individuals, and are of two sorts, one of which is finely fibred and the other more coarsely fibred.

10. Endochondral osteogenesis continues for some distance into the metaphysis and thus consolidates the trabeculae.

11. As growth in length slows down, there is a deposition of endosteal lictor-bundle (and later lamellar) bone, which occurs throughout the medullary cavity.

12. The endosteal bone formed during the egg-laying cycle is irregularly woven.

13. The relation of periosteal osteogenesis to linear growth, and the significance of perivascular osteogenesis are discussed.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Transverse section of a portion of the diaphysis of the femur from a 15-day-old cockere showing the vascular spaces in the bone, and the neonatal sheath separating the inner bone formed during embryonic life from the outer bone formed after hatching. Long's method. (× 120.)

Fig. 2. Transverse section of a portion of the diaphysis of the femur from a cockerel aged 29 days. Note the trabeculae of bone forming under the periosteum and the conversion of these into

perivascular structures. The neonatal sheath has disappeared by this stage. Long's method ($\times 81$.)

Fig. 3. Transverse section of a portion of the diaphysis of the femur from a cockerel aged 246 days. Showing from the internal surface outwards, the lamellar endosteal bone separated by a cement line from the woven bone containing numerous primary osteones and a single secondary osteone, and the outer densely fibred lictor-bundle bone. Long's method. ($\times 125$.)

Plate 2

Fig. 4. Longitudinal section of the diaphysis of the femur from a 17-day-old embryonic fowl. Showing the perichondrial bone immediately adjacent to the hypertrophic cartilage of the diaphysis. The finely fibred early embryonic woven bone is seen to be continuous with the perichondrial bone. Long's method. ($\times 780$.)

Fig. 5. Longitudinal section of the diaphysis of the femur from a cockerel aged 15 days (compare with fig. 1). Note the appearance of the neonatal sheath and the obliquely running fibre bundles whose courses are interrupted by the sheath. Long's method. ($\times 270$.)

Fig. 6. Longitudinal section of the peripheral portion of the diaphysis of the femur from a cockerel aged 155 days, showing the lictor-bundle bone. Long's method. ($\times 780$.)

Fig. 7. Transverse section of the peripheral portion diaphysis of the femur from a cockerel aged 379 days (compare with fig. 6) showing the lictor-bundle bone. Long's method. ($\times 330$.)

Fig. 8. Longitudinal sections of the diaphysis of the femur from a cockerel aged 246 days, showing the elastic fibres present on the outer part of the cortex. Weigert's elastin stain. ($\times 180$.)

Fig. 9. Transverse section of the diaphysis of the femur from an adult hen showing the elastic fibres. Compare with figs. 3 and 8. Weigert's elastin stain. ($\times 180$.)

Fig. 10. Transverse sections of the diaphysis of the femur from a cockerel aged 29 days, showing the perivascular arrangement of fibres. Compare with fig. 2, which shows the more rapidly growing surface. Long's method. ($\times 330$).

Plate 3

Fig. 11. As fig. 10 at greater magnification showing the hyaline lining to the vascular spaces. Long's method. ($\times 1692$.)

Fig. 12. Longitudinal section of the metaphysis of the femur from a cockerel aged 29 days, showing the endochondral bone which contains numerous osteocytes and surrounds islands of unresorbed hypertrophic cartilage. Long's method. ($\times 780$.)

Fig. 13. Transverse section of the diaphysis of the femur from a cockerel aged 379 days, showing a large secondary osteone with a lamellar fibrous structure. Long's method. ($\times 330$.)

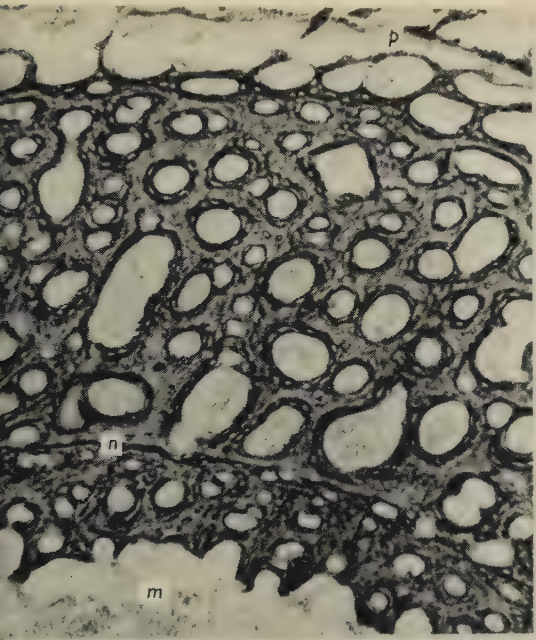
Fig. 14. Transverse section of the diaphysis of the femur from a cockerel aged 249 days, showing a secondary osteone containing coarse fibre bundles running in the longitudinal axis of the osteone. Note the numerous coarse tendon fibre bundles in the surrounding woven bone. Long's method. ($\times 360$.)

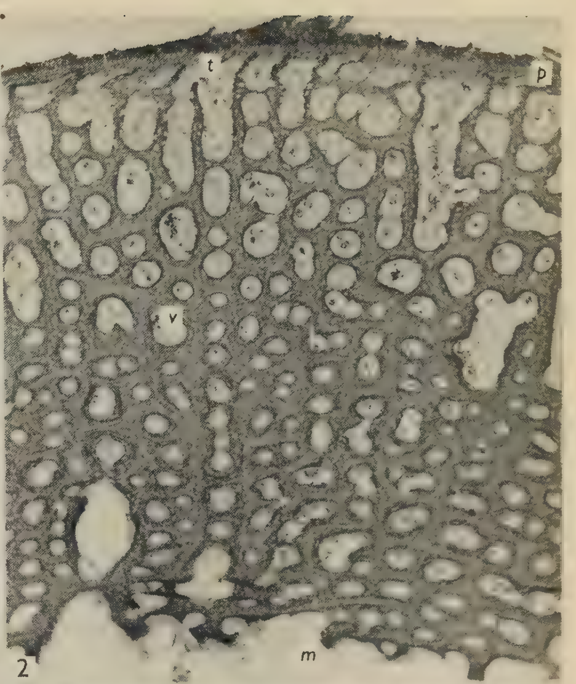
Fig. 15. Transverse section of the diaphysis of the femur from a cockerel aged 249 days, showing the lamellar fibrous structure of the endosteal bone. Long's method. ($\times 780$.)

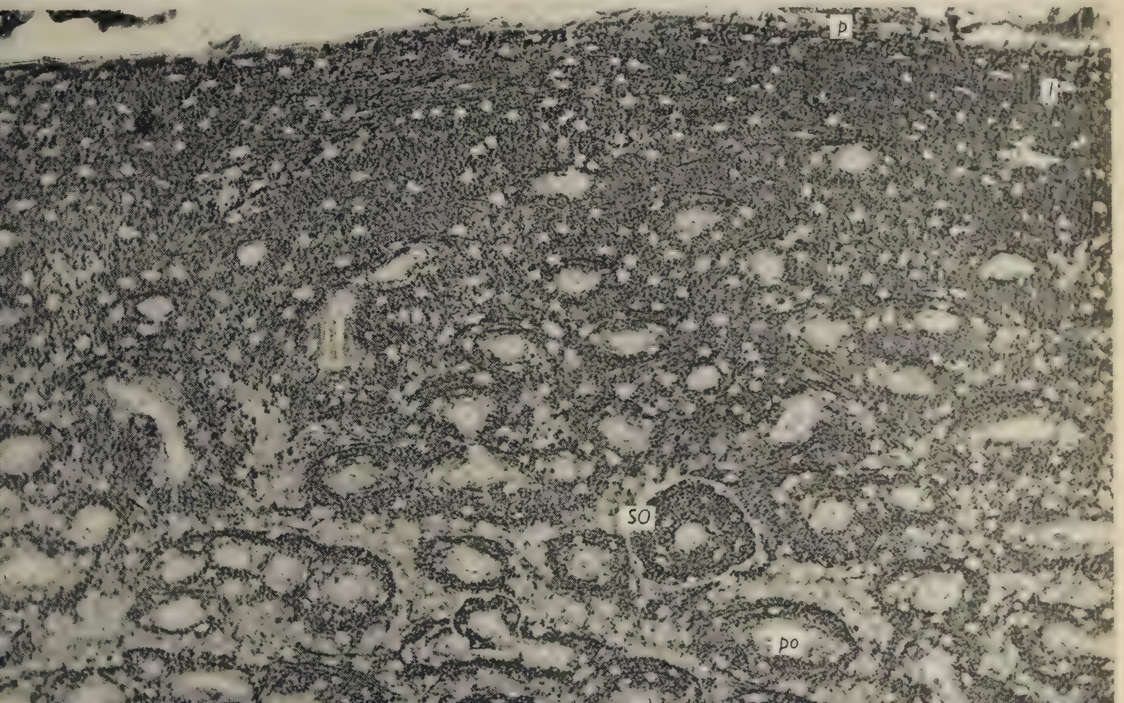
Fig. 16. Transverse section of the diaphysis of the femur from an adult hen, showing the fibrous structure of the intramedullary trabeculae of 'egg-laying' bone. Long's method. ($\times 330$.)

Key to lettering

c = cement line; e = endosteal bone; el = elastic fibres; h = hypertrophic cartilage; hl = hyaline lining; l = lictor-bundle bone; la = lacuna; m = marrow cavity; n = neonatal sheath; p = fibrous periosteum; pb = perichondrial bone; po = primary osteone; so = secondary osteone; t = subperiosteal trabecula; v = vascular space; w = woven bone.

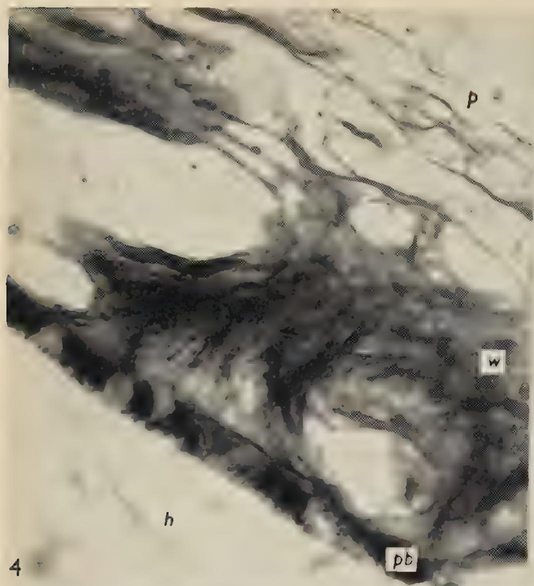


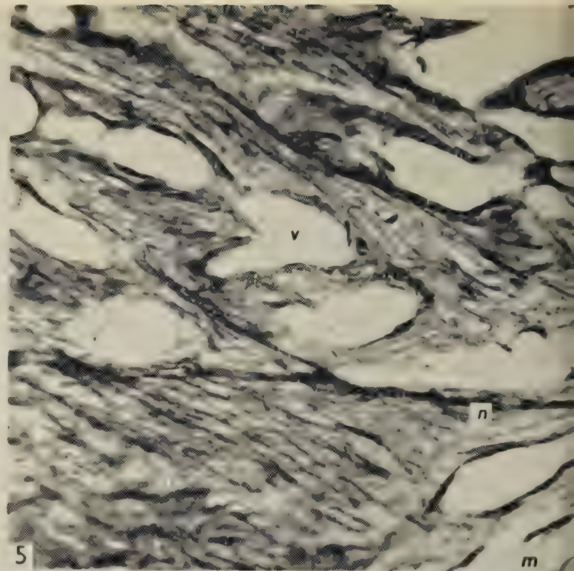


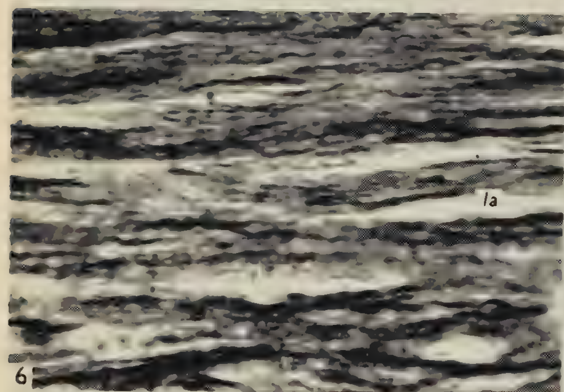


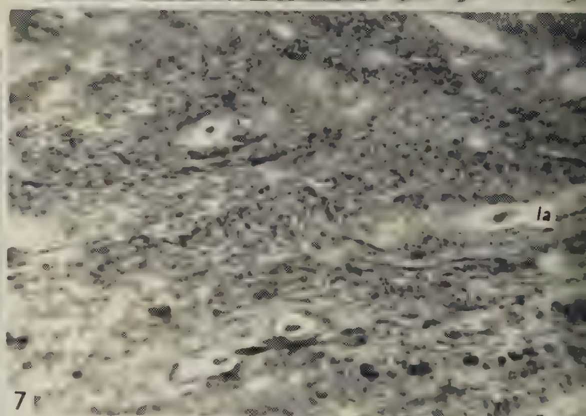
PRATT—EFFECT OF AGE ON THE ARRANGEMENT OF FIBRES

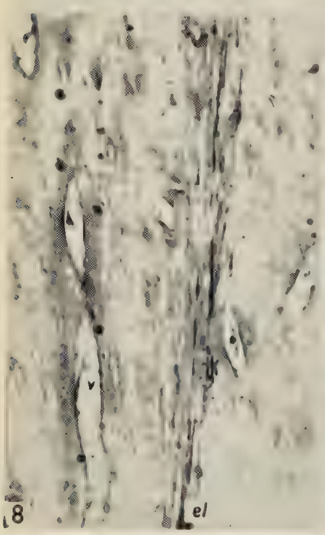
(*Facing p.* 122)

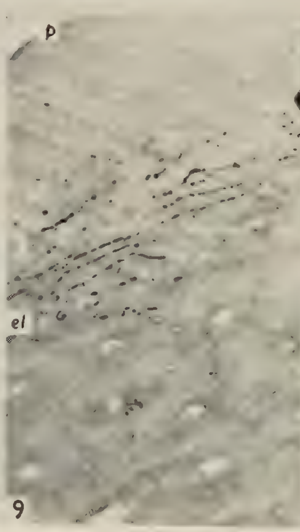


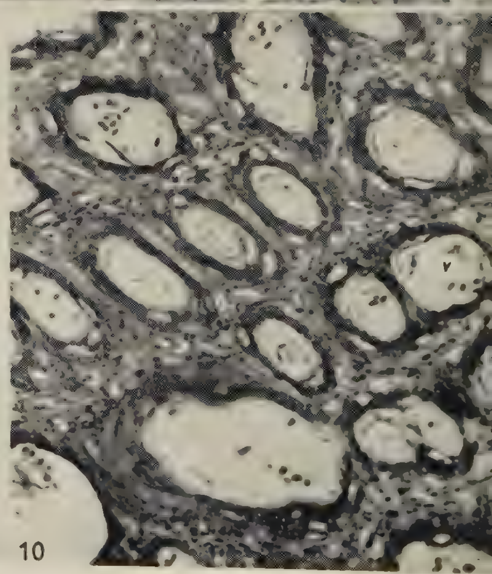












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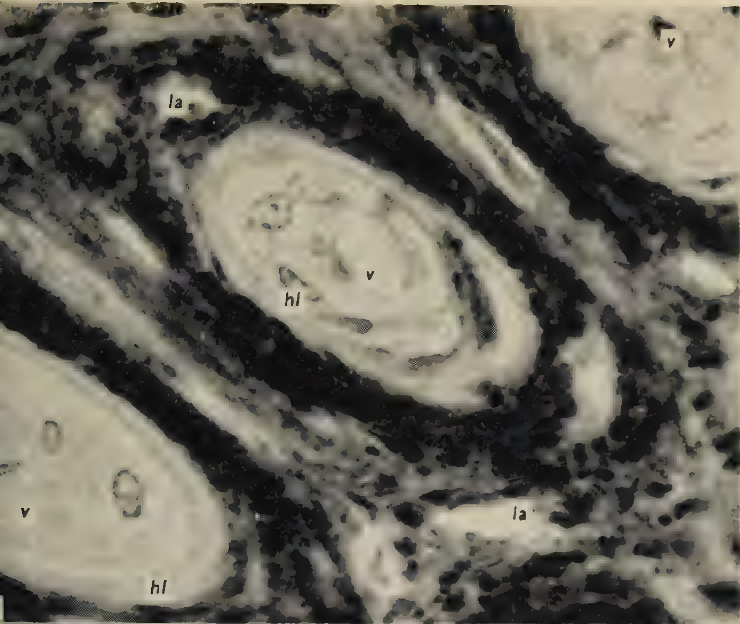

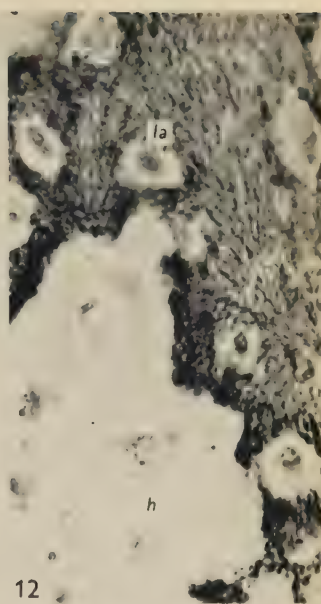

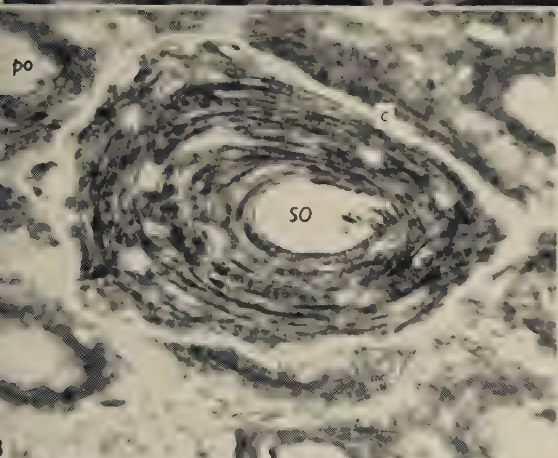

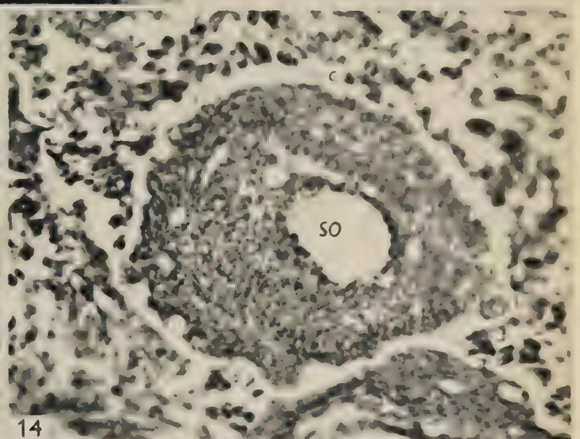

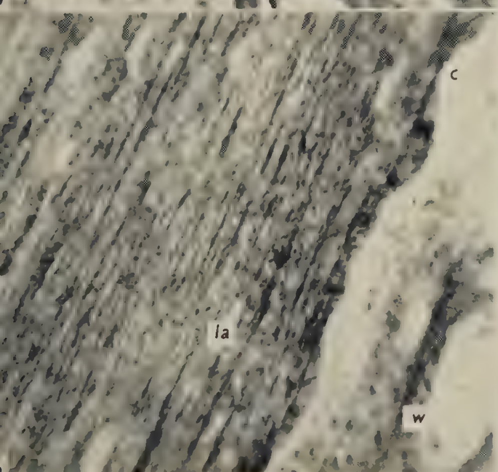

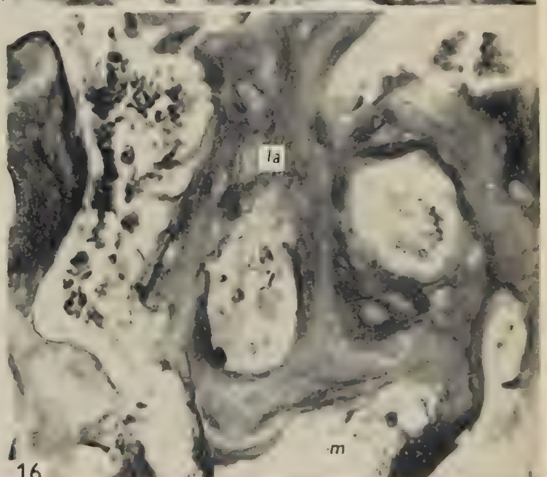

PRATT—Effect of age on the arrangement of fibres

THE TWO HEADS OF FLEXOR POLLICIS BREVIS

By M. H. DAY and J. R. NAPIER

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The flexor pollicis brevis muscle has been the subject of controversy for over a century, and reference to modern text-books originating in Great Britain and France indicates that the matter is still unresolved.

The recent editions of most British texts* describe the muscle as having a *single head* attached proximally to the crest of the trapezium and adjacent flexor retinaculum and distally to the radial sesamoid and radial side of the proximal phalanx of the thumb. In current French texts (Paturet, 1951; Rouvière, 1954) the muscle is described as having *two heads*—superficial and deep; the superficial head corresponds to the whole muscle of modern British texts, and the deep head corresponds to the fasciculus that, passing deep to the tendon of flexor pollicis longus, reaches the radial sesamoid; this fasciculus is generally regarded in this country as part of the oblique head of adductor pollicis. This disparity, which was discussed at length by Wood Jones (1942), still leads to misunderstandings, particularly with regard to nerve supply, and thus to confusion in the diagnosis and treatment of median and ulnar nerve injuries.

The earliest adequate account of flexor pollicis brevis in the literature appears to be that of Albinus (1749), who described two heads, outer and inner; the outer arose from the front of the carpus and passed to the radial sesamoid (the deep head of Cruveilhier), and the inner passed from the front of the carpus to the ulnar sesamoid (the oblique head of adductor pollicis of modern anatomists). The muscle now known to British anatomists as the flexor brevis was regarded by Albinus as a second short abductor. This interpretation of the morphology was followed in general by many continental anatomists during the next hundred years.

In 1841, Henle introduced another element, the first palmar interosseus, while still following the teaching of Albinus with regard to flexor pollicis brevis. Later, Bischoff (1870) stated that the interosseus primus volaris of Henle was the 'true deep head' of flexor brevis. Bischoff's view was quoted by Brooks (1885–6), who described flexor brevis as having outer and inner heads; he left the question of the existence of 'Bischoff's true deep head', i.e. the interosseus primus volaris of Henle, completely open.

It is of interest to review the changing attitudes in British anatomical literature from the early nineteenth century. It is clear that both Flemming's and Brooks's views (see below) have influenced British anatomists who have adopted Flemming's superficial head and Brooks's (Bischoff's) deep head, and relegated Cruveilhier's deep head to the adductor pollicis complex. This definition of the muscle which first

* E.g. *Gray's Anatomy* (1958), Cunningham's *Textbook of Anatomy* (1951), Buchanan's *Manual of Anatomy* (1949), a textbook of Anatomy by Lockhart, Hamilton and Fyfe (1959). *Anatomy* by R. J. Last (1959) is an exception; this author advocates including with the flexor pollicis brevis all muscles inserted on the radial sesamoid.

appeared in the 13th edition of *Gray's Anatomy* (1893) persisted until the 27th edition when the deep head (1st palmar interosseus) was returned to the interosseus layer to which it morphologically belongs. This change was foreshadowed in the 22nd edition (1923), where it was admitted that the deep head of flexor brevis 'was sometimes described as the first volar interosseus'.

Table 1. *Some British views of flexor pollicis brevis* (1834–1958)

Text-book	Superficial head	Deep head
1834, *Quain's Anatomy*	Superficial head of Cruveilhier	No deep head
1858, *Gray's Anatomy*, 1st ed.	,, ,, ,,	Oblique head of A.P.
1893, *Gray's Anatomy*, 13th ed.	,, ,, ,,	1st palmar interosseus of Henle
1936, *Gray's Anatomy*, 27th ed.	,, ,, ,,	No deep head
1958, *Gray's Anatomy*, 32nd ed.	,, ,, ,,	No deep head

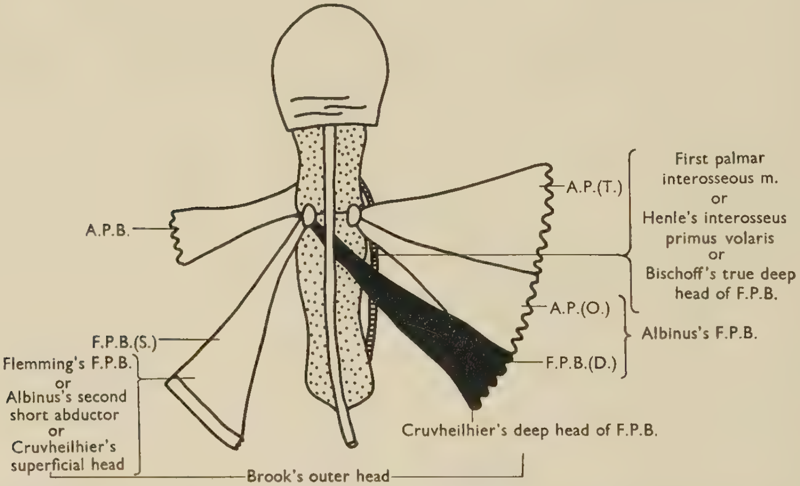

Fig. 1. The left thumb and associated muscles illustrating the views of the various authorities mentioned in the text. The opponens pollicis has been omitted for reasons of clarity. The deep head of flexor pollicis brevis is shown in black.

The French view of flexor pollicis brevis as stated by Cruveilhier (1834) has been followed, with few exceptions, to the present day.

This communication presents evidence, based on a re-examination of the form and nerve supply of the muscle, that the current British description is inadequate and to recommend that the classic view of the French school should be adopted.

RESULTS

Form

The form of the flexor pollicis brevis has been studied in a series of sixty-five hands from thirty-six male and female cadavers. Both hands have been dissected in twenty-nine subjects, of whom twenty-seven showed symmetrical findings.

Type I (Fig. 2 I). Fifty-three hands (twenty-three paired, seven unpaired)

The flexor pollicis brevis had two heads; the superficial head arose from the crest of the trapezium and the adjacent flexor retinaculum and the deep head from the trapezoid and capitate bones and the palmar ligaments of the distal row of carpal bones. The deep head passed deep to flexor pollicis longus tendon and united with the superficial head on the radial sesamoid bone and the base of the proximal phalanx of the thumb.

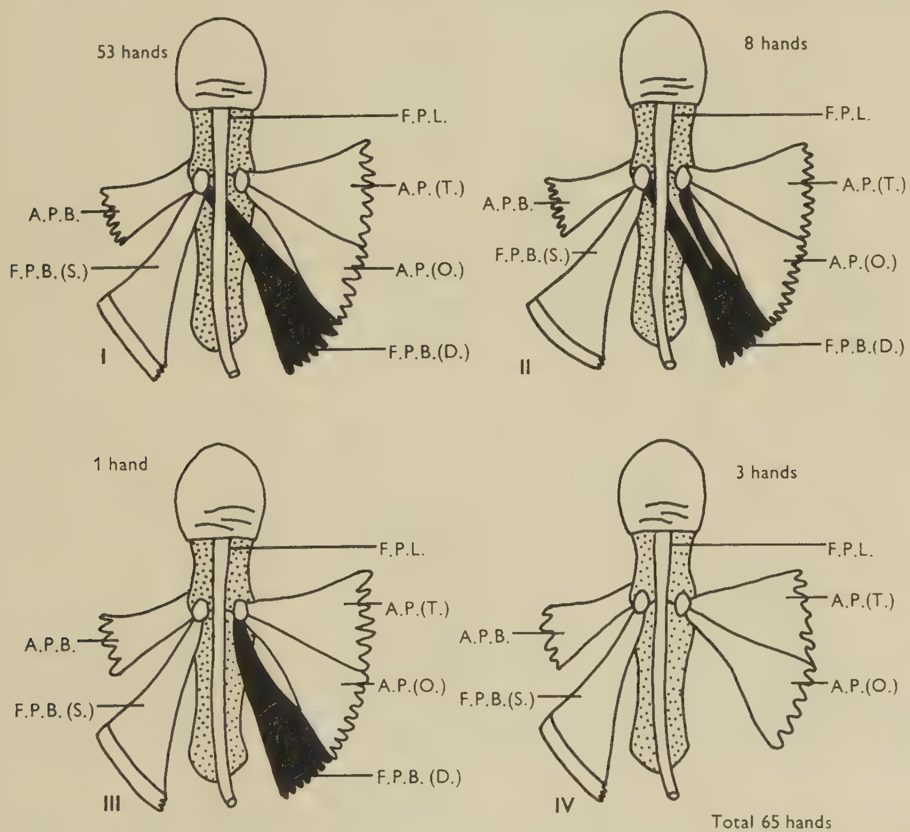

Fig. 2. Diagrams of left thumb showing the four types (I, II, III, IV) of flexor pollicis brevis muscle.

Type II (Fig. 2 II). Eight hands (three paired, two unpaired)

The deep head had two slips: one was attached to the radial sesamoid, the other to the ulnar sesamoid.

Type III (Fig. 2 III). One hand.

The deep head was represented by a slip attached distally to the ulnar sesamoid.

Type IV (Fig. 2 IV). Three hands (one paired, one unpaired)

The deep head was absent.

A deep head thus was present in sixty-two out of a total of sixty-five hands (twenty-seven paired, eleven unpaired). In three cases only was a deep head absent.

In all instances where a deep head was present, a definite connective tissue plane existed between it and the oblique head of adductor pollicis. In several instances the distal attachment of the deep head had spread from the radial sesamoid to reach the anterior aspect of the proximal phalanx.

Nerve supply

It was only possible to study the nerve supply of the two heads of the flexor pollicis brevis in thirty of the sixty-five dissections. The findings are summarized in Table 2.

Table 2. *The nerve supply of flexor pollicis brevis in* 30 *specimens*

Head	Median nerve supply alone	Ulnar nerve supply alone	Median and ulnar nerve supply	Total observations
Superficial	17	6	7	30
Deep	3	16	5	24

It was not possible to establish the nerve supply of the deep head in six cases. The totals given above include ten hands (four paired, two unpaired) in which a special dissection of the nerve supply was carried out following the method described by Brooks (1885–6). A dissecting microscope was used where necessary. These findings, which probably should be regarded as the more critical, are summarized separately in Table 3.

Table 3. *The nerve supply in* 10 *specimens dissected by Brooke's method*

Head	Median nerve supply alone	Ulnar nerve supply alone	Median and ulnar nerve supply	Total observations
Superficial	6	2	2	10
Deep	3	5	2	10

These figures suggest that, while variation of the nerve supply is common, there is a tendency for the superficial head to be supplied by the median nerve (twenty-four out of thirty dissections) and for the deep head to be supplied by the ulnar (twenty-one out of twenty-four dissections).

DISCUSSION

The cause of the controversy surrounding this muscle is largely a semantic one. The main stumbling block has been the variety of synonyms employed to designate the two parts of the muscle; for example, Brooks's outer head, the outer head of Albinus and the superficial head of Cruveilhier are not equivalent as might be supposed. This has led to confusion in later publications, particularly in regard to the nerve supply of the two heads (see below). An attempt has been made to trace the history of the controversy. Cruveilhier (1834) described the muscle as having two heads, a superficial arising from flexor retinaculum and the adjacent portion of trapezium, and a deep head arising from the anterior carpal ligament, the trapezoid and the capitate.* Cruveilhier's view of the muscle has appeared consistently in French

* For the sake of clarity throughout the discussion that follows the deep head of the flexor pollicis will be referred to as the deep head of Cruveilhier to distinguish it from the various deep heads described by other authors.

texts up to the present day (Sappey, 1876; Poirier & Charpy, 1912; Paturet, 1951; Rouvière, 1954). This accordance contrasts sharply with the changeable views to be found in British texts over the past hundred years.

Whether the nerve supply of the flexor pollicis brevis is to be regarded as median, or as median and ulnar, clearly depends on whether or not the deep head of Cruveilhier is accepted as part of the muscle. The literature of this muscle shows there is considerable confusion amongst anatomists and clinicians on this point. The account given by Brooks (1885–6) of the innervation of the *outer* head is probably the best available, but it must be remembered that Brooks's *outer* head was equivalent to *both* the deep and the superficial heads of Cruveilhier. Brooks found that the two heads of Cruveilhier were innervated by both median and ulnar nerves in nineteen out of thirty-one of his dissections; and that of the remaining twelve dissections, five showed that the two heads of Cruveilhier were innervated by the deep branch of the ulnar and seven by the motor branch of the median. Brooks's findings with regard to his outer head are summarized in Table 4. It is clear from

Table 4. *Variations in the nerve supply of flexor pollicis brevis in man* (Brooks, 1885–6)

Head	Median nerve supply alone	Ulnar nerve supply alone	Median and ulnar nerve supply	Total observations
Outer head (i.e. deep and superficial heads of Cruveilhier)	7	5	19	31

Brooks's figures, and from our own findings (Table 2), that the whole muscle (the superficial and deep heads of Cruveilhier) usually receives a dual innervation. Unfortunately Brooks provided no details of his series of nineteen cases in which the muscle was supplied by median and ulnar nerves. Thus, although dual innervation of the superficial and deep heads individually cannot be excluded, there is no anatomical justification for stating that a dual nerve supply to the superficial head of Cruveilhier alone is the commonest finding. Our own observations have shown that in only seven cases out of thirty did the superficial head of Cruveilhier have a dual innervation. Rowntree (1949), although he recognized the existence of a deep head, did not take it into account in his conclusions; he stated that the flexor pollicis brevis *usually* had a dual nerve supply. This statement was not supported by his figures, which showed that in only thirty-eight of 226 cases was there clinical evidence of dual supply. He admitted this inconsistency, but explained it on the grounds that the existence of dual innervation might well remain clinically unobserved. Rowntree did not attempt to distinguish between dual supply of *the whole muscle* (deep and superficial heads) and dual supply of the *superficial head* only; indeed such a distinction would be almost impossible to make by clinical means alone. His findings, thus, are clearly open to the misinterpretation that the *superficial head* of flexor pollicis brevis has a dual nerve supply, especially by authorities who only recognize one head—the superficial one (*Gray's Anatomy*, 32nd edition).

This paper has demonstrated that the existence of a deep head is the common arrangement in the human hand (sixty-one out of sixty-five cases). The issue now clearly depends upon whether the deep head should be regarded as part of adductor

pollicis, or as part of flexor pollicis brevis. The problem can be approached from several viewpoints—the purely morphological, the phylogenetic and the functional.

Morphologically, in man, the muscle slip in question appears to be as much part of adductor pollicis as it is of flexor pollicis brevis. Generally speaking, it is customary to give more weight to the insertion than to the origin in determining muscle homologies, but as the reason for this is purely functional, it will be considered under that heading. The question of nerve supply is clearly a point to consider in determining homologies, and it was undoubtedly on these grounds that Flemming (1887), influenced by the work of Kleinenberg (1872) and Gegenbaur (1874) on neuro-muscular specificity, held that only that portion of the short flexor of the thumb supplied by the median nerve could be correctly termed the flexor pollicis brevis. It was presumably also on these grounds that British anatomists finally dropped all reference to a deep head (*Gray's Anatomy*, 25th edition).

The comparative studies of Haines (1935), and the experimental work of Lovell (1931), Detwiler (1936), and Piatt (1939, 1940) on nerve regeneration in the limbs of *Amblystoma*, summarized by Straus (1946), make it clear that the concept of nerve-muscle specificity, in the sense that Fürbringer (1888) postulated, is no longer tenable. It seems that there is, therefore, no valid reason for excluding the deep head of Cruveilhier from the flexor pollicis brevis complex purely on the grounds of nerve supply. Indeed the evidence of variability, which this report and that of Brooks (1885–6) make clear, argues strongly in favour of including the deep head, for it suggests that this head has migrated in phylogeny from an original situation where it was associated with ulnar-innervated musculature, to the position in which it is now commonly found in man. Haines (1935) and later Straus (1946*a*) pointed out that a muscle which migrates will eventually take its nerve supply from the most convenient source. Brooks (1885–6), on the other hand, put forward the theory of a muscle bridge by which means the migration of a muscle provides a route whereby a nerve can cross from one territory to an adjacent one. It seems likely that both processes are involved. Brooks's theory offers an explanation for the occasional extension of the ulnar nerve into the superficial head, and Haines's (1935) theory provides a reasonable explanation for the occasional extension of the median nerve into the deep head.

Phylogenetic considerations. The close relation of the deep head at its origin to the oblique head of adductor pollicis makes it likely that it is a derivative of the contrahentes layer of the mammalian intrinsic palmar musculature (Brooks, 1885–6; McMurrich, 1902–03; Haines, 1935) from which the adductor pollicis of man has also been derived.

The comparative studies of Cunningham (1878), Brooks (1885–6), and others, suggest strongly that the insertion of the deep head has migrated from the ulnar to the radial sesamoid. It is generally agreed (Cunningham, 1878; McMurrich, 1902–03; Howell, 1936; Haines, 1950) that the superficial head is derived from the flexor brevis superficialis muscle group of the generalized reptilian hand. In *Tejus* (Ribbing, 1907) and *Uromastix* (Haines, 1950) the muscle of the radial digit is composed of two heads passing to either side of the proximal phalanx of the thumb at their insertion. This arrangement is found in many generalized marsupial forms (Young, 1879). The ulnar head has presumably been suppressed in eutherian

mammals, and has been replaced, as Brooks (1885–6) suggested, by the radial slip arising from the contrahentes layer.

Functional considerations. There is little doubt that the action of the deep head of the short flexor is identical to all intents and purposes with that of the superficial head (Wood Jones, 1942); the direction which the fibres of the two heads bear to the carpo-metacarpal joint of the thumb varies little. Both superficial and deep heads act as flexors of the carpo-metacarpal joint and the metacarpo-phalangeal joint of the *adducted* thumb. When the thumb is *abducted* by the abductor pollicis brevis the two heads provide the motive power for the movement of circumduction by which the abducted thumb is pulled into flexion and medial rotation.

The phylogenetic significance of the migration of the deep head from an ulnar to a radial attachment appears to be related in Primates to the acquisition of true opposability of the thumb (M. H. Day & J. R. Napier, in preparation).

There seems on all counts therefore little justification for regarding the deep head of Cruveilhier as part of the adductor pollicis complex; morphologically, phylogenetically and functionally this slip forms part of the flexor pollicis brevis.

SUMMARY

A discrepancy exists between modern British and French texts with regard to the definition of the flexor pollicis brevis muscle. An historical survey of the literature reflects the profound differences of opinion that have existed for well over a century.

Evidence is presented, based upon sixty-five dissections, that flexor pollicis brevis consists of a superficial head arising from the crest of the trapezium and adjacent flexor retinaculum, and a deep head arising from the trapezoid and capitate bones and the palmar ligaments of the distal row of carpal bones. Both heads unite on the radial sesamoid bone. A variable nerve supply was found in the series of thirty dissections, the commonest pattern observed being a superficial head supplied by the median nerve and a deep head supplied by the ulnar nerve.

The significance of the deep head is discussed in terms of morphology, phylogeny and function. It is concluded that there is adequate evidence to regard the deep head of Cruveilhier as part of the flexor pollicis brevis muscle.

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THE EFFECTS OF THYROID DEFICIENCY ON THE GROWTH OF THE RAT SKULL

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INTRODUCTION

It has been shown (Eayrs & Taylor, 1951) that the brains of hypothyroid rats grow proportionately less in length than they do in width or height. Eayrs (1953) has suggested that growth changes of the brain may be secondary to a disproportionate growth of brain and endocranium resulting from growth changes in the skull similar to those following hypophysectomy in infancy (Asling, Walker, Simpson, Li & Evans, 1952). In the course of a previous investigation into the effect of thyroid deficiency on brain growth (Horn, 1955) it was noticed that the shape of the skulls of experimental animals, as seen on radiographs, was different from that of the normal controls. The present study was undertaken to investigate in more detail the nature of these changes and to determine the extent to which they parallel those reported to follow hypophysectomy.

MATERIAL AND METHODS

A total of fifty-five rats of the Wistar strain were used, drawn from twenty-two litters. Twenty-five experimental animals were each injected with 100 μC of carrier-free ^{131}I (Goldberg & Chaikoff, 1949) immediately after birth, as previously described (Horn & Hess, 1958). Each experimental animal was paired with a normal littermate control of the same sex, and five such pairs killed at 10, 15, 20, 30 and 40 days of age. The precise age at death for a given pair was selected, at the time of birth, on a random basis. If an experimental or control animal died before the appointed day the pair were discarded, unless a littermate of appropriate sex and treatment was available, and a fresh pair substituted. Not all pairs in any set of five were of the same sex. Besides these fifty animals, five more, four male and one female, taken from separate litters, were killed immediately after birth.

Immediately after death radiographs were taken of each rat in three positions (Ford & Horn, 1959) so as to display the bone shadows outlining the maximum length, width and height of the endocranial cavity. The three positions were:

(1) Lying ventrally on the plate with a cotton thread passing over and pressing on the back of the neck, to keep the cranial base parallel to the plate.

(2) Lying on the side with the two external auditory meatuses on the same vertical line.

(3) Lying ventrally on the plate with the thorax raised so that the nostrils were on the plate and in the same vertical plane as the external auditory meatuses (thus giving a postero-anterior view of the skull).

Measurements were made direct from the radiographs using calipers with a vernier scale measuring to 0·1 mm. With a tube-plate distance of 3 ft. used for taking

Table 1. *Mean values and standard deviations of dimensions measured*

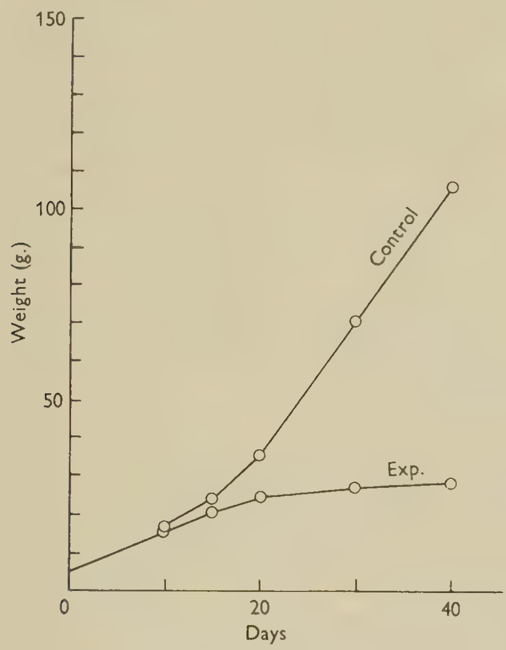
Age (days)...		Birth		10		15		20		30		40	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Weight	Exp.			16·00	3·13	**20·50**	4·86	**24·34**	3·15	**27·56**	5·68	**27·92**	4·64
	Contr.	5·26	0·63	16·46	1·25	**24·60**	4·65	**35·94**	1·00	**70·96**	8·14	**106·64**	5·33
Nasopelvic length	Exp.			64·60	5·26	**70·14**	5·06	**74·96**	3·55	**82·46**	5·35	**84·30**	4·21
	Contr.	44·74	1·49	66·48	3·04	**78·24**	5·03	**89·92**	3·42	**118·00**	3·20	**136·76**	2·82
Skull length	Exp.			23·74	1·92	**26·24**	1·53	**27·76**	1·38	**29·40**	1·22	**30·04**	0·64
	Contr.	14·78	0·35	24·20	1·41	**28·06**	1·47	**30 54**	0·80	**34·76**	0·62	**37·02**	0·44
Endocranial length	Exp.			16·78	1·33	*18·70*	0·78	*19·16*	0·71	**19·42**	0·75	**19·50**	0·23
	Contr.	11·22	0·48	17·04	0·92	*19·36*	0·92	*20·50*	0·33	**21·64**	0·41	**22·50**	0·34
Endocranial width	Exp.			12·38	0·74	13·40	0·36	13·94	0·21	14·04	0·24	*14·02*	0·35
	Contr.	8·12	0·32	12·38	0·47	13·71	0·33	13·91	0·39	14·34	0·24	*14·85*	0·26
Endocranial height	Exp.			*8·26*	0·45	9·16	0·21	9·08	0·17	9·02	0·25	*8·84*	0·46
	Contr.	5·16	0·16	*7·88*	0·32	8·92	0·20	8·92	0·27	9·22	0·21	*9·50*	0·19
Bizygomatic diameter	Exp.			13·80	0·97	*14·84*	0·57	*15·14*	0·46	**15·78**	0·66	**16·16**	0·40
	Contr.	9·36	0·41	14·02	0·59	*15·36*	0·61	*16·10*	0·36	**18·18**	0·32	**19·48**	0·20
Cribriform plate width	Exp.			5·00	0·18	5·30	0·17	5·26	0·21	5·28	0·16	5·28	0·12
	Contr.	3·72	0·10	4·92	0·17	5·10	0·17	5·16	0·08	5·18	0·12	5·14	0·05

Italicized figures indicate a significant difference with $P < 0·05$.
Figures in heavy type indicate a significant difference with $P < 0·01$.

the radiographs, errors due to magnification or distortion of the image amounted to about 1 %. This was of the same order of magnitude as the limits of accuracy imposed by use of the vernier scale, and was therefore discounted.

The measurements taken on the skull are indicated in Pl. 1, and the results are given in Table 1. Naso-pelvic length (distance from most anterior point of skull to most posterior point on pelvis) was also measured.

Paired t-tests were used on the data derived from each age group to determine the level of significance of any differences that were observed.



Text-fig. 1. Mean weights of experimental (Exp.) and control groups of rats at 10, 15, 20, 30 and 40 days.

RESULTS

The destructive effects of ^{131}I on the thyroid glands, as shown by their histological appearance, were similar to those which have previously been described (Horn, 1955). Besides these glandular changes the difference in weights between experimental and control animals provided further evidence of hypothyroidism. The weight of the experimental animals was significantly less than that of the normal controls from the age of 15 days onwards (Table 1). In addition, the increase in weight of the hypothyroid animals was very small after the age of 20 days (Fig. 1). These weight changes are similar to those described by Scow & Simpson (1945) in rats whose thyroids and parathyroids have been removed at birth.

The hypothyroid animals were shorter at 15 days and thereafter, as shown by their reduced naso-pelvic lengths, compared with their controls. The over-all length of the skull was reduced in the experimental animals from this age onwards. In both

of these dimensions the differences between experimental and control animals increased greatly with age.

The endocranial length showed changes with age similar to those in the over-all skull length but the endocranial width and height did not. The mean width of the endocranial cavity in thyroid-deficient animals was not significantly different from that of the controls up to and including the 30th day after birth. At 40 days it was slightly, though significantly ($P < 0{\cdot}05$) smaller. On the other hand the mean bizygomatic width of the experimental animals was progressively, and significantly, less than that of the controls from the 15th day onwards. The mean endocranial height, in contrast to all other skull dimensions, was slightly greater in experimental than in control animals aged 10, 15 and 20 days. The difference is statistically significant ($P < 0{\cdot}05$) in the 10-day-old group. At 30 and 40 days of age the mean endocranial height was slightly less in the hypothyroid animals compared with their controls. Pl. 1 shows a typical hypothyroid cranium at 20 days compared with a normal control.

The mean width of the cribriform plate, a dimension which in the normal rat does not increase after 15 days (Ford & Horn, 1959) was not reduced by thyroid deficiency; indeed, this dimension was consistently greater in the experimental animal than in the controls. This difference was not statistically significant in any one age group, but when all experimental animals of 15 days and over were grouped together and compared with their similarly grouped controls the mean values differed significantly ($P < 0{\cdot}05$).

DISCUSSION

Eayrs & Taylor (1951) measured certain dimensions of the brains of hypothyroid rats. These measurements may be correlated with the present series of measurements on the endocranial cavity. It was felt that little would be added by measuring the brains of the animals in the present series, since it would have been extremely difficult to take measurements from points on the brain which corresponded precisely to those on the skull, and since deformation of the soft unfixed brain or shrinkage, due to fixation would produce distortion of unknown magnitude. The shape of the brain of hypothyroid animals at 24 days (Eayrs & Taylor, 1951) is, in general, similar to that of the endocranial cavity in all the ^{131}I-treated animals of the present series beyond the age of 15 days; width and height of both brain and cranial cavity are much less affected by hypothyroidism than length which is significantly reduced compared with the corresponding dimensions of the control animals. The altered brain shape of cretinous animals is thus accompanied by a similar alteration in shape of the endocranial cavity.

There are at least three hypotheses which may be formulated concerning the relationship between brain and skull growth in hypothyroidism. It is possible that (*a*) the shape of the endocranial cavity and the shape of the brain are determined independently, (*b*) the shape of the endocranial cavity is the principal factor which determines the shape of the brain, and (*c*) the shape of the brain is the principal factor which determines the shape of the endocranial cavity.

The length of the endocranial cavity is largely determined by growth of bones at the cranial base and the width and height by growth of the bones of the vault.

Bones of the axial part of the cranial base ossify in cartilage and those of the vault in membrane. Differential interference, by thyroid deficiency, with endochondral ossification would thus result in a skull reduced in length but little affected in width or height—a shape, that is, similar to that found in cretinous rats. However, the work of Todd, Wharton & Todd (1938) suggests that endochondral ossification is less affected than intramembranous ossification in thyroidectomized animals. These workers found that the growth of the maxilla and premaxilla of thyroidectomized sheep was more affected than that of the mandible. The maxilla and premaxilla grow predominantly in membrane, like the bones of the cranial vault, while much of the growth of the mandible takes place in secondary cartilage of the mandibular condyle. Thus it would be reasonable to predict that the endocranial dimensions dependent upon intramembranous growth would be smaller than those dependent on endochondral growth. This would give an endocranial cavity longer, lower and narrower than in normal animals. The fact that this expectation is not fulfilled, since it is growth in length which is most retarded in the cretins, suggests the operation of some local factor in determining skull shape, for example, the endocranial contents, and thus weakens both hypotheses (*a*) and (*b*) while being consistent with (*c*).

The only observation that we are aware of, which appears not to be consistent with the view that the endocranial contents, to some extent at least, determine the shape of the endocranial cavity, is that described by Asling *et al.* (1952). These workers found that in rats hypophysectomized 6 days after birth, brain weight was not affected up to 80 days of age. However, the skull failed to gain adequately in length, though it was considered that there was a greater than normal growth in width and height. In some of these animals there was increased intracranial pressure as evidenced by herniation of the medulla and cerebellum out of the foramen magnum. In the course of hypophysectomy the spheno-occipital synchondrosis was destroyed. Injection of growth hormone to the hypophysectomized animal re-established growth in length of the skull, the growth occurring at the spheno-presphenoid synchondrosis. Although growth could take place at this synchondrosis in the untreated hypophysectomized animals, destruction of the spheno-occipital synchondrosis is likely to interfere seriously with growth in skull length, and therefore to reduce the capacity of the skull to grow in length at the same rate as the brain, which was compressed in consequence. Hence provided that the skull retains its capacity to respond to changes in brain shape, i.e. provided that the sites of growth are intact, the hypothesis that brain growth is the principal factor in determining the shape of the endocranial cavity is not contradicted by the work of Asling *et al.* (1952). This hypothesis is the simplest one, which accounts for the observations made in the present study.

SUMMARY

1. A series of hypothyroid rats, together with their normal littermates as controls, were killed at various ages from birth to 40 days of age. Hypothyroidism was induced by injection of ^{131}I. Immediately after death radiographs were taken from which measurements of certain skull and bodily dimensions were made.

2. Endocranial dimensions were unequally affected by hypothyroidism: compared

with control dimensions endocranial length was significantly reduced at and after 15 days, but endocranial width and height were not significantly reduced up to and including 30 days of age.

3. A number of hypotheses to account for these observations were considered, and it was concluded that the simplest of these and the one which most satisfactorily accounts for the findings was that changes in growth of the brain are of primary importance in determining endocranial growth in hypothyroidism.

We wish to thank Prof. J. D. Boyd for his helpful advice, and Mr J. A. F. Fozzard for the many hours he has spent in obtaining the excellent series of radiographs for us.

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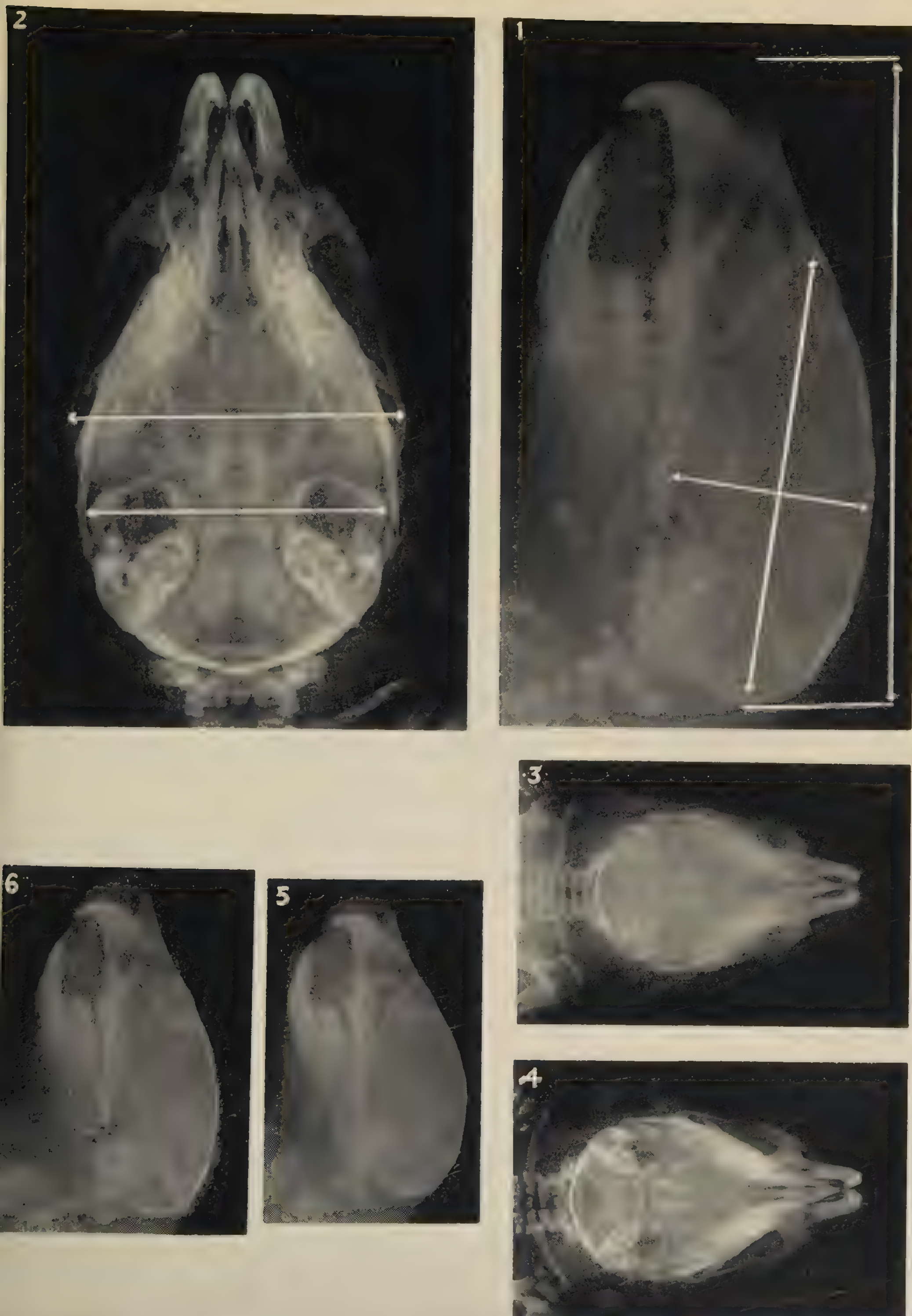
EXPLANATION OF PLATE

Fig. 1. Lateral X-ray of normal rat head at 20 days, to show how the measurements of skull length, endocranial length and endocranial height were taken. ×4.

Fig. 2. Supero-inferior X-ray of normal rat head of 20 days, to show how measurements of endocranial width and bizygomatic width were taken. ×4.

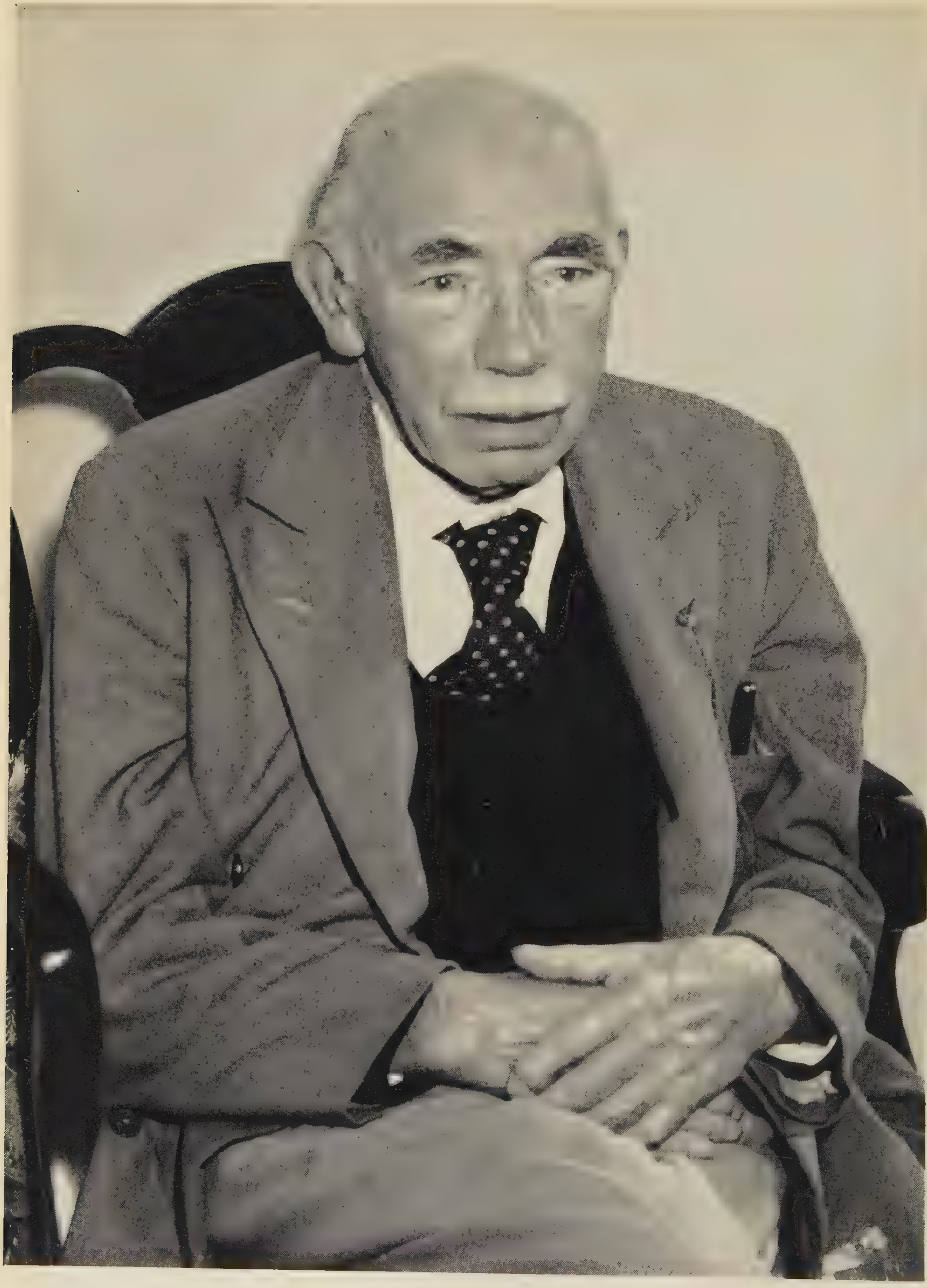
Figs. 3, 4. Supero-inferior X-rays of hypothyroid (Fig. 3) and normal (Fig. 4) littermate rats of 20 days, for comparison. ×2.

Figs. 5, 6. Lateral X-rays of the same hypothyroid (Fig. 5) and normal (Fig. 6) littermates, showing the shortening and doming of the cranium characteristic of hypothyroidism. ×2.



HORN AND FORD—Growth of the rat skull

(Facing p. 136)



THOMAS BAILLIE JOHNSTON, C.B.E., M.D.

(*Facing p.* 137)

IN MEMORIAM

THOMAS BAILLIE JOHNSTON, C.B.E., M.D.

Professor Emeritus T. B. Johnston died at Guy's Hospital on 8 October 1960 at the age of 77 years. The son of Major A. Johnston he was born and bred in Edinburgh, where he received his education at George Watson's College and the University of Edinburgh. His first attempt at the school-leaving certificate was not particularly distinguished, but he ended in third place in the higher section. Though his family was keen that he should join the Indian Civil Service, he sat for and was awarded jointly the Vans Dunlop Scholarship and qualified M.B., Ch.B. with first-class honours in 1906. He obtained the M.D. degree in 1938. In 1907 Johnston was appointed a demonstrator and in 1911 a lecturer in Anatomy at Edinburgh. He moved to University College in 1914 to work under George Dancer Thane and was for a short time Sub-dean of the Medical Faculty. His work was interrupted by the First World War and from 1916 to 1918 he held a Temporary Commission in the R.A.M.C. and saw active service in France and Italy. He was mentioned in Dispatches in 1918.

In 1919 Johnston was appointed to the first Chair in Anatomy at Guy's Hospital Medical School. Here his administrative ability was quickly recognized and in 1920 he was appointed Dean of the School, a position which he held until 1937. When he took over the post of Dean the School premises were poor and inadequate, but the number of students was swelled by ex-service men, of whom many were married and often in financial difficulty. It was with these students that T.B., as he was popularly known, made his mark as a teacher. During his tenure of the Deanship new buildings to house most of the Medical School departments were constructed, a new constitution for the School was drawn up and a Preliminary Clinical Period was introduced into the courses. As recreation T.B. spent much of his leisure time at Huntercombe Manor Golf Club where he was popularly known as 'the Dean' and where he formed a close friendship with Lord Nuffield who he was able to interest in the finances of Guy's Hospital. In 1938 Johnston, having resigned as Dean, took up the post of Superintendent of the hospital, a somewhat difficult and unenviable task at the time for it was deeply in debt. However, the onset of the Second World War and the take over by the Ministry of Health after the war resolved this difficulty. As superintendent Johnston was the liaison between the medical staff and the governors and acted as spokesman for the staff. Whilst he held the post of superintendent T. B. Johnston's appointment as Professor was part-time. It was intended to have a second Chair of Anatomy but this was never filled and it was only in 1948 that he was succeeded in the Chair by the late James Whillis.

During the Second World War Johnston was Group Officer for No. 10 Sector with its headquarters at Orpington. At the end of the war he returned to Guy's to rebuild and reorganize a much-damaged hospital; the building of the new surgical block, which reached its final stages last year, was largely due to his efforts and it will

remain as a memorial of his unceasing work on behalf of Guy's Hospital. On his retirement from the Chair he was elected Professor Emeritus and on his retirement from the post of superintendent a member of the new Board of Governors. He was awarded the C.B.E. in 1948.

In the Anatomical Society Johnston was the second oldest member, having joined in 1912, in the same year as his old friend, T. B. Layton. He was a Vice-President of the Society and, after his retirement, a Life Member. He represented British anatomists at the International Congress in Milan in 1936 and was, until a year ago, a representative of the Society on the International Nomenclature Sub-committee. With A. F. Dixon and E. B. Jamieson he prepared and presented to the Society in 1933 the revised anatomical terminology, now known as the Birmingham Revision. Jointly with G. A. G. Mitchell, he was secretary of the International Nomenclature Committee which presented its report in 1955 at the International Anatomical Congress in Paris. He spent a great deal of time and energy in the preparation of these terminologies and it is surprising how he found time for all this in the midst of his manifold duties.

Johnston published several papers in the *Journal*, mainly on embryological topics but with excursions into neurology and comparative anatomy. However, it is as editor of anatomical textbooks that he will be best remembered. He was the author of *Medical Applied Anatomy*, published in 1915; *A Manual of Surgical Anatomy*. with L. Beesly; and *A Synopsis of Regional Anatomy*, which reached its eighth edition in 1955. He was editor of *Gray's Anatomy*, at first alone, later with James Whillis and then with Francis Davies and myself. Overall, he was editor of *Gray* for nearly thirty years, a record which is unlikely to be surpassed. His one ambition was to edit the centenary (thirty-second) edition which appeared in 1958. Originally he was recommended to the publishers by F. G. Parsons for the post of editor to work with and succeed R. Howden. When Howden was in London for a G.M.C. meeting in 1928, he met Johnston to discuss the proposition, but their discussions led to the unexpected resignation of Howden from the post of editor, leaving T.B. to produce the twenty-fourth edition on his own. This appeared in 1930 and in it Johnston had entirely rewritten the embryology section. As editor he worked with four generations of the publishing firm of Longmans Green and with three generations of the printing firm of Maclehose. As a colleague, T.B. was all one could wish. He was helpful and tolerant of the inexperienced, but was insistent on a high standard of work and conservative in his outlook.

T.B. will be remembered not only as editor of *Gray*, but as a versatile teacher of generations of medical students and as a great administrator. His success in this sprang from his charm and ability to get on with his colleagues whilst maintaining the highest of standards.

D. V. DAVIES

REVIEWS

Erläuterndes anatomisches Wörterbuch. By T. Donath. (Pp. 538; $7.00.) Budapest: Verlag Medicina. 1960.

This is an arranged list of the equivalent terms from the B.N.A. (1895), J.N.A. (1935) and P.N.A. (1955) together with some unofficial names not included in any of these terminologies but in current use. It does not include the most recent additions and alterations adopted at the International Anatomical Congress held in New York in 1960. There is in addition a large explanatory section based on the B.N.A., together with an extensive bibliography and a detailed index. This book should be in the possession of all serious-minded anatomists and available in all anatomical, and indeed medical, libraries. The author is to be complimented on the very considerable task which he has accomplished so accurately.

D. V. DAVIES

Anatomischer Atlas des menschlichen Körpers. By F. Kiss and J. Szentagothai. (Vols. I–III, pp. 639, 792 plates, $24.00.) 7th edition. Budapest: Verlag Medicina. 1960.

This atlas is arranged systematically; vol. I includes osteology, arthrology and myology, vol. II splanchnology and heart and ductless glands, and vol. III the nervous system, the peripheral vascular system and the special sense organs. The illustrations are mainly of dissections with some sections, especially in the part on the nervous system, and a few diagrams. In general the illustrations are often not very detailed and in the case of the osteology the finer bone details are lacking and the amount of labelling is restricted. Many of the illustrations are in colour and are attractively produced on art paper. Each volume is of convenient flat demi quarto size. The terminology used is the Basel Nomina Anatomica with a few additional unofficial and eponymous terms which are in common use. Accompanying vol. III is a small booklet wherein the equivalent terms of the B.N.A., J.N.A. and P.N.A. are neatly set out together with the page and figure numbers on which each structure is illustrated. The authors and publishers are to be congratulated on the production of an attractive, useful and accurate, if not very detailed, atlas. Since this review was written an eighth edition has been published in English.

D. V. DAVIES

Some Papers on the Cerebral Cortex. By Gerhardt von Bonin. (Pp. xxiv + 396; 42 figures; 92*s.*) Springfield, Illinois, U.S.A.: Charles C. Thomas. 1960.

This book is a collection of twelve papers of well-known workers on the anatomy and physiology of the cerebral cortex. With the exception of one paper in English by A. S. F. Leyton and C. S. Sherrington they are translations of writings in French and German. The author, well known for his contributions to knowledge on this subject, has deliberately omitted publications by English and American authors because these are readily available in the original. The temptation to include the one English paper proved, not surprisingly, too strong and terminates in 1917 a story told in these chronologically arranged papers beginning in 1823.

The choice of the material included is of course the author's personal preference and one cannot doubt that it will prove invaluable to students of this subject. Many readers, knowledgeable about this topic, will doubtless have their own opinions about the choice of the material and might feel that the book could be improved with modification. However, it is unlikely that these critics will agree with one another, and the book should prove

a happy compromise wherein all readers will achieve some satisfaction. It is, however, without doubt, unfortunate that the material contained in this book concludes in 1917; had it been continued into modern times the work of such authors as the Vogts, von Economo and Foerster, to quote a few examples, might profitably have been included.

The introduction to this book is admirable. In addition to providing a historical review of the subject with brief biographical sketches of many famous neurological workers of the past it also contains a most valuable critical review. As an anatomist, however, issue must be taken with the first paragraph of the introduction wherein it is stated: 'What might be termed the anatomical era has come to a close, and the physiological era has begun.' It would be as well to remind the author of this erroneous statement that because the physiologist has only at last begun to develop a glimmer of a concept of cortical function cortical structure has not ceased to matter. He will ultimately be forced to recognize a truth appreciated by anatomists for many years that structure and function are intertwined and there still remains much for the anatomist to study in the cortex before the physiologist can correctly unravel its function. However, this small introductory irritation must not be allowed to prejudice the otherwise highly commendable large remainder of a book which all those interested in the subject should possess.

W. HEWITT

The Process of Aging in the Nervous System. Edited by JAMES E. BIRREN, HENRY A. IMUS and WILLIAM F. WINDLE. (Pp. xii + 224; 43 figures; 52*s.* 6*d.*) Illinois: Charles C. Thomas; Oxford: Blackwell Scientific Publications. 1959.

This is the record of the proceedings of a symposium held in 1957 at Bethseda, Maryland, U.S.A. Thirty-two contributors participated and discussed, at 'Round-Table' conferences, the eleven papers which were presented. The foreword consists of the introductory address delivered by the Director of the Bethseda Institute.

A key problem of the discussions was the interpretation of 'aging'. In his opening address Pearce Bailey defines this as 'the biological changes taking place in the nervous system with the passage of time'; a reasonable definition finding support in the *Oxford Dictionary*. However, as Dr Pearce Bailey develops his theme one becomes aware that his confidence in his definition is unsure and his later remarks become confusing. The ranges covered by each contributor varied widely and some concerned themselves with a limited time span.

The papers cannot be discussed individually as each was a strictly personal communication subsequently discussed at the 'Round-Table' conference. All possessed some merit as would be expected from contributors so well qualified to participate. Noteworthy among the contributions was that by Harold E. Himwich on 'The Biochemistry of the Nervous System in relation to the Process of Aging', wherein many biochemical studies on the whole brain are considered over the whole life span, providing much valuable and interesting data.

One is not informed whether the audience at this symposium was confined to the thirty-two contributors, but it is most likely that others were present.

One purpose of symposia is that those unfamiliar with a subject and lacking the time to plough through the ever-increasing volume of literature can hear a resumé of a subject. After listening to topics presented by so-called experts in the field, the inexpert should be enabled to broaden their view of the knowledge they have gained, by participation in the subsequent discussion. The 'Round-Table' discussions are most interesting to read and one gains a much wider view of the topics in the papers previously presented. However, this form of discussion if confined only to participants familiar with the subject has not the appeal or value of an open discussion to which the entire audience contributes. It does, in fact, remind one of a closed shop policy in science and it is to be hoped that this form of discussion will not find support in this country.

The publishers of this book can be well satisfied with its production, but its content is its disappointing feature, for it largely consists of tit-bits of information. I regret I could

recommend this book only to libraries and the few interested in this subject who may find its content and considerable bibliography a useful starting-point for a fruitful field of research. Its real value, perhaps, is the revelation of just how little is known about aging in the nervous system.

W. HEWITT

The Antecedents of Man. By Sir WILFRID LE GROS CLARK, F.R.S. (Pp. 374+vii; 152 figures; 21*s*.) Edinburgh: University Press. 1960.

The Foundations of Human Evolution. By Sir WILFRID LE GROS CLARK, F.R.S. (Pp. 74+8 figures. $1.00.) Oregon: University Press. 1959.

The first and larger of these two publications, though reminiscent of the author's earlier classic *The Early Forerunners of Man* (1934), is a wholly new work, much more catholic in range—an entirely new review, analysis and presentation of the extensive data of the field of present-day Primatology, with particular attention to and emphasis upon the evolutionary emergence of hominid forms. To this end the relevant findings of human and comparative anatomy, of palaeontology, of taxonomy and of genetics have been laid under tribute and critical scrutiny made of an impressive wealth of morphological data, the resultant inferences and conclusions being presented with logical and masterly objectivity. The style of presentation is Huxleyan in its clarity, restraint and forceful persuasiveness, proffering much recondite matter with an Addisonian simplicity, and thus extremely helpful to the non-specialist reader. But only the professional primatologist will fully appreciate how extensive and how intimate an acquaintance with both factual evidence and the literature is so frequently disguised under cover of succinctly informative statements.

The initial chapter (on the evolutionary process and the Primates) provides a necessary background for what is to follow and proclaims the high standard to be expected throughout the book. This chapter, an acute and invaluable analysis of principles and procedure in the modern fields of comparative anatomy, embryology and palaeontology, provides a wholesome and educative introduction to the subsequent subject-matter, and will be read profitably by the general and by the specialist reader alike.

The second chapter (the Primates in space and time) summarizes admirably the anatomical characters and the palaeontological record of each Primate group from the Anthropoidea to the Lemurs and the Tree Shrews: a model of accurate and clear presentation, it must prove invaluable to the non-specialist in palaeontology.

Chapters 3–9 consider seriatim, in respect of each component Primate group, such evidence as to evolution and taxonomic inter-relationship as is provided by the dentition, the skull, the limbs, the brain, the special senses and the digestive and reproductive systems: this evidence is, consistently, submitted with commendable restraint and objectivity, the facts being allowed to proclaim their own significance without bias or persuasion on the author's part. And in these chapters, again, perhaps only those who have tilled some corner of the primate evolutionary field will be in a position to appreciate (with admiration) the extent of the evidence reviewed, the labour thereby involved and the acumen wherewith the value of that evidence is presented.

The final chapter which discusses the evolutionary radiations of the several Primate groups will prove pleasantly helpful to anatomist and palaeontologist equally, both for its synthesis and interpretation of the available evidence and for the inferences drawn with such commendable caution from the fossil record.

The book is a repository of information and an exemplar of balanced deduction: it bears throughout the unmistakable impress of its author's unrivalled familiarity with Primatology in all its aspects, as also of his sustained (and successful) efforts to rescue hominid evolution from the subjectivity (and too often, alas, the factionalism) of the personal approach and to establish the subject on the soundly objective basis of a scientific discipline.

The volume will prove an authoritative companion to all interested or engaged in the study of Primate evolution—both human and non-human—and an indispensable adjunct

to the bookshelf of the zoologist, the anatomist and the palaeontologist. It is a dependable and comprehensive introduction to that morphological background against which alone hominid evolution is to be intelligibly interpreted and understood.

Material evidence, whether fossil or recent, is frequently patient of more than one interpretation, and therefore the criticism may be made that views and interpretations which, in specific instances, differ from the author's, are not accorded a fuller recognition. One also wonders whether the invocation of genetic change to account for morphological disparities and trends is, though logically convenient, as explanatory as commonly supposed.

The Edinburgh University Press merit congratulation upon so excellent a production, with such attractively clear type and such effective illustrations, and so modestly priced.

The second and briefer publication represents in book form the Condon Lectures for 1959 delivered under the auspices of the Oregon State System of Higher Education in celebration of the centenary of Darwin's *Origin of Species*. The introductory lecture is devoted to Darwinian prognostications and to theoretical considerations vital to appreciation of Primate phylogeny: succeeding lectures are essentially a review of the status and intrinsic affinities of the component groups which constitute the total Primate assemblage. Thus the relationship is discussed between Man and the anthropoid apes, between these apes and the Catarrhini, between the Catarrhini and the Platyrrhini, between the Prosimii and the Tree Shrews and so on. The terminal lecture outlines briefly the evolutionary morphological sequences to be recognized as existing between the earliest and lowliest Primates and the most recent and highest. Tree Shrews and Lemurs are accounted Primates: the evidence for this procedure is briefly reviewed, but will not perhaps carry conviction to all. The ancestors of the Hominidae, as yet unknown from any direct fossil evidence, are convincingly assigned to the Miocene era. Of the illustrations provided, the reconstructed external features of *Australopithecus* and *Pithecanthropus* are perhaps somewhat too fancifully 'humanized'.

A. J. E. CAVE

Die Altersveränderungen der Halswirbelsäule. By U. Ecklin (with foreword by Prof. G. Töndury). (Pp. 79+vii and bibl.; DM. 28.) Springer-Verlag. 1960.

Just over a century ago Luschka wrote a treatise on the semiarthroses (*Halbgelenke*) between the bodies of the cervical vertebrae, 2nd to 7th inclusive. Dr Ecklin's book on this topic is based upon further work started by Prof. Töndury, carried on by Dr J. Nick and completed by himself. It covers the whole span of human life from the womb to old age, considering the origin of and changes in these joints both ontogenetically and phylogenetically. The cervical vertebrae are considered against the background of the whole column, the cervical semiarthroses are viewed against the background of the cervical vertebrae. The whole thing is a first-rate exercise in human endomorphology, in both space and time.

The author's principal thesis can be paraphrased by saying that the nutatory movements of the lower five cervical vertebrae are provided for by the development of lateral bursae within the intervertebral discs, not outside them as Luschka thought. As age advances these lateral bursae may extend medially and fuse with each other. The bursae begin deep to the uncinate processes of the upper surface of a vertebral body, and may show meniscus-like partitions. They constitute effectively a pair of *uncovertebral joints*, the uncinate processes being processes of the upper (not the lower) parts of the neural arches. Although students in our islands have been taught something about these joints for decades the said joints have not yet received any mention in the official terminologies. Dr Ecklin's treatise should lead to a rectification of this omission. The whole work is a valuable addition to the arthrology of the vertebral column and deserves a place in both anatomical and radiological libraries. It was rightly awarded the chief prize from the special prize fund of the University of Zürich.

M. A. MACCONAILL

Cytology and Evolution. By W. N. WILLMER, M.A., Sc.D. (Pp. 430; $10.00.) New York, London: Academic Press.

This is a most stimulating text which should have a wide, if rather specialized appeal. The first part of the book is concerned with the classification of cell types, based largely on the activities of cells in tissue culture but also calling upon many of the associated disciplines of cytochemistry, biochemistry and electron microscopy. The author postulates a phylogenetic tree of cell types starting with an 'epitheliocyte', with its two subfamilies of 'mechanocytes' and 'amoebocytes'. From these three postulated basic types cell families are suggested, and on page 226 a possible genealogical tree of cell families in vertebrates is put forward. This simple summary does not do justice to the wealth of material and literature that Dr Willmer includes.

Among the important collateral discussion throughout this part of the book there runs the concept of cytological homoiostasis, stressing the importance of the cellular function in relation to the maintenance of its environment. This theme is continued in the next part of the book in a detailed discussion of the possible ontogenetic and phylogenetic origins of secretory cells, under the heading of coelomoducts, nephridia, kidneys and gonads.

A further section of the book deals with the origins of the retina in vertebrates; again discussed from the point of view of cellular origins, and there is a final chapter giving the author's concepts of the factors to be kept in mind in investigating cellular activity.

From the above short synopsis it will be seen that the author's own description of this as an unusual book is not out of place. Dr Willmer describes the book both as 'a text-book' and as 'a series of essays'. He is to be congratulated on having accomplished a most difficult task in bringing together information from so many different disciplines. He admits that the book is 'much less concerned with the description of observed data than it is with their interpretation'. There is, however, a wealth of data and references and, whilst one may not agree with the author's interpretations, these are provocative and useful.

This is a unique book, which will provide interesting and stimulating reading for all biologists.

G. CAUSEY

Reticuloendothelial Structure and Function. Ed. by JOHN H. HELLER. (Pp. iii + 473; 160 figures; $12.00.) New York: The Ronald Press Co.

This is the report on the Symposium held by the International Society for Research on the Reticuloendothelial System at Rapallo in 1958. The book contains the text of thirty-two papers covering a very wide field of related investigations and draws very widely on different disciplines and nations. The reticuloendothelial system is interpreted in its widest functional sense, as can be seen from the groups of papers on antibody response and synthesis, leucocyte response, immunological response and the relationship of the RES to neoplasia and hyperlipemia.

It is difficult in such a large group of papers with varying standards and including so many different disciplines to select special articles for mention, but it is worth pointing out that only the first article is purely morphological, and in this article Dr Fresen accepts the RES as a functional entity, combining storage and phagocytosis with humoral defence mechanisms and metabolism and then attempts, both by optical and electron microscopic studies, to define the cellular entities that should be included. All workers will not agree with his assumption of reticular cells being distinguishable by their fibrillary structure. This lack of unanimity is clearly brought out in the report of the discussion on this contribution at the Congress.

Prof. Loutit discusses 'Immunological tolerance in radiation-chimeras' concisely and clearly, Prof. Nichol and Dr Bilbey give further reports on 'The effect of various steroids on the phagocytic activity of the RES'. These articles are selected as being the only contributions by British authors. The text as a whole will form an essential reference for

those interested in the special fields discussed, but will have a greater appeal to the pathologist and microbiologist than to the morphologist. The book is excellently produced and well illustrated. Some of the discussion has been reported and there is a useful index.

G. CAUSEY

Das innere Lymphgefäßsystem der Organe. By FERENC RÉNYI-VÁMOS. (Pp. 448; 278 illustrations.) Published by the Hungarian Academy of Sciences. 1960.

This monograph is the German translation of a Hungarian manuscript and is based on clinical and experimental research carried out over a period of ten years. It deals mainly with the intrinsic lymph vessels of viscera; the pathways outside the organs, and the lymph nodes are seldom considered.

The general part of the book (120 pages) deals with the techniques of demonstrating lymphatics and discusses general anatomy, physiology and pathology of the lymphatic system. The special part deals with lymph vessels of individual organs, giving particular consideration to their role in various pathological conditions. The kidney, ureter and bladder have received additional attention from the author who is a urologist.

The book is well written and well illustrated, mostly by means of original microphotographs, some of which are in colour. The bibliography is excellent and contains over 500 references. There is a subject index as well as a separate author's index.

This monograph is essential for research workers interested in vascular problems, and it can be warmly recommended to clinicians who will find useful information on a variety of clinical problems.

N. CAUNA

The Surgical Anatomy of the Bronchovascular Segments. By WILLIAM E. BLOOMER, AVERILL A. LIEBOW and MILTON R. HALES. (Pp. vii+273; 208 figures; £6. 12*s*. 0*d*.) Oxford: Blackwell Scientific Publications. 1960.

This book gives a description of the bronchial trees and their associated blood vessels from the main bronchi to the subsegmental divisions of the lobar bronchi. The information is based on multicoloured plastic corrosion casts of fifty lungs. An aim of the book is to describe how the anatomical information is used in surgical operations. The result of the study of the casts is magnificently recorded by black and white and coloured illustrations. One can have only the greatest admiration for the infinte pains and care taken to produce these records.

The need of applying all this detail to surgery is obscure. Supposing that it is possible to remember it, it could produce confusion. There are easier and more effective techniques available, and techniques which are based on the operative field as found at the time. It is also unfortunately true that tuberculous inflammation, for instance, often does not keep to clear-cut segmental areas. It is of educational interest to a surgeon, however, to know how much there is to know about the bronchial segments.

G. KENT HARRISON

Vertebrate Dissection. By WARREN F. WALKER. 2nd ed. (Pp. xii+340; 69 references; 104 figures. 30*s*.) W. B. Saunders Company Ltd.

There is much to be said for and against the author's claim that the systemic approach, which he uses in this book, is the best method of dissecting representative types. I think this dissection manual is only suitable for advanced zoological classes because it is essential that the student has some general knowledge of the structure of animals before attempting systemic dissections. Also I feel that there is always the danger of overlooking the interrelationships of the different systems in any one animal.

The manual is divided into eleven chapters plus appendices. The first deals with the Lower Chordates (e.g. *Balanoglossus*, *Saccoglossus*, *Molgula* and *Amphioxus*) and acts as

a very useful introduction. The second is devoted to a Primitive Vertebrate (*Petromyzon marinus*), so in this chapter the author has stuck to the more traditional methods. The third—the Evolution and External Anatomy of Vertebrates—is a little too theoretical (this can also be said of many of the other chapters); for example, he gives a phylogenetic tree which I think is unnecessary in a 'Vertebrate Dissection'. The remaining eight chapters are taken up with a comparison of the different systems in dogfish (*Squalus*), mudpuppy (*Necturus*), cat (*Felis*) and rabbit (*Lepus*) together with a few diagrams of other types (e.g. some bones of reptiles and brain of sheep) where it is necessary to demonstrate some point.

The scope of this review does not permit comments on all the systems described so I will confine my remarks to two. The chapter on the muscular system is very extensive and in addition to the general description and comparison the author has given a table showing 'the probable homologies'. The other very useful chapter is the one dealing with 'the Coelom and the Digestive and Respiratory Systems'. It enables the student to trace the many changes associated with the evolution of a terrestrial life.

All systems are fully described, illustrated and compared. If the student has time (this is rarely the case in a practical class) to read all the detail given and carry out the dissection at the same time, then he will acquire a mass of information from this manual.

The appendix which deals with the preparation of specimens includes methods for embalming and injecting, the importance of which is not always realized by the student.

A. G. HAMILTON

Primatologia. Vol. II, 5 Lieferung. Edited by H. HOFER, A. H. SCHULTZ and D. STARCK. (iv + 74 pp. 35 figures.) 20 Swiss francs. Basel: S. Karger, 1960.

The publishers of this excellent and informative series have decided to issue future volumes in the form of separate Lieferungen. The present section deals with the auditory organ and comprises two chapters, one by C. J. Werner of Leipzig dealing with the middle and internal ear and the other, by Lasinski of Gdansk, with the external ear. The middle and internal ear show comparatively little change through the Primate series, but the external organ exhibits wide diversification and accordingly lends itself to more extensive treatment. Nevertheless, Werners's section receives very thorough treatment and includes a number of original observations. Lasinski's chapter gives an excellent résumé of earlier literature reinforced with personal examination of 250 ears representing the majority of the recognized genera.

W. C. OSMAN HILL

Ciba Foundation Symposium on Congenital Malformations. Edited by G. E. W. WOLSTENHOLME and CECILIA M. O'CONNOR. (Pp. xii + 308, 45*s*.). London: J. and A. Churchill, Ltd. 1960.

The post-war period has witnessed a reawakening of interest in teratology. At the turn of the century workers in this field were in the main concerned with purely descriptive accounts of monstrosities. With surgical advances in this field, even these accounts are proving inadequate and it is interesting to learn that further volumes are now being added to the well-known but incomplete work of Schwalbe. Experimental embryology and advances in embryonic and foetal physiology have shifted the emphasis. It is over forty years since Stockard popularized the concept of critical periods and it would appear that his postulates are still acceptable. Though most of the early work has been done on the lower vertebrates, the principles apply equally to mammalian development. Not only does an organ develop from a definitely located anlage but also during a fixed period in development. A multiplicity of factors, genetical or environmental, may produce similar abnormalities if allowed to act at a comparable stage in development, and what is now called for is an elucidation of the precise mechanism of the primary derangement.

A symposium was held at the Ciba Foundation headquarters in January 1960, at which some twenty or more workers—all authorities in the teratological field—presented the results of their work and their views as to the mechanism of the abnormalities. Their papers and the discussions that followed them, together with a general discussion at the end of the meeting, have been collected together in this book. They include papers on the genetical and environmental aspects of the problem including the effects of various deficiencies in the mother and the action of antibiotics. All are clearly written, on the whole concise and are worth reading. Together they give a good indication of the trends of modern research in this field. Curiously, however, the name of Stockard does not appear within the covers of the volume which should be available not only to those interested in teratology but also to those primarily concerned with the normal anatomy and physiology of the embryo and foetus.

The Ciba Foundation is to be praised for the manner in which it fosters medical research and gives hospitality to delegates to symposia such as these. The Foundation not only provides the opportunity for scientists to meet and discuss the problems which they have in common, but also makes the proceedings of the meetings available to others who cannot be present to participate in the symposia.

D. V. DAVIES

The Jaws and Teeth of Primates. By B. W. WARWICK JAMES. (Pp. xii+328, 250 figures, £5. 5*s*. 0*d*.). London: Pitman Medical Publishing Co. 1960.

There can be nothing but admiration for Dr Warwick James who, at well over 70 years of age, is still continuing the scientific work he loves and has pursued over the last half century.

This volume is, in the main, an atlas of the teeth and jaws of primates, almost entirely from the collection at the British Museum (Natural History). The photographs are technically excellent. There are three views of each specimen, the first being a lateral view with the jaws and teeth almost in occlusion, the second a slightly oblique view of the upper jaw and teeth from below and the third an oblique view of the lower jaw and teeth from above. Together they show the details of dental morphology to the best advantage and will serve as a useful record, particularly for those who have no access to the rarer forms, most of which are depicted in this volume. Each set of photographs is accompanied by brief notes of the genus and a short account of the dental anatomy.

In addition to the photographic record, there is an elementary account of primate teeth, jaws and temporomandibular joint, together with an introductory account of primates in which some of their principal characters are described. The classification of primates is also included. Unfortunately the terminology of the dental cusps and a discussion of their homologies are not considered, although there are adequate references to the authoritative works on these topics.

Here and there are a few errors and inconsistencies, but these are not sufficiently serious to mar the volume, which should provide a useful, accurate and readily available source of information on primate dental anatomy for students and research workers alike. The author is to be congratulated on his efforts as are the publishers on the quality of the illustrations.

D. V. DAVIES

The Discovery of Reflexes. By Professor E. G. T. LIDDELL. (Pp. 173; 23 figures; 30*s*.) Oxford University Press. 1960.

When neurophysiology may to some appear synonymous with oscilloscope-watching, it is proper that we be reminded how present basic ideas have grown up. A few workers combining clear vision and painstaking method with the use of simple yet effective tools cleared away much of the dead wood of the past. Of such was Sherrington. In this short work, Prof. Liddell has set out to show clearly the magnitude of Sherrington's contribution.

He has done this by placing it in relation to the past, recalling first the growth, somewhat haphazard, of factual knowledge about nervous system and muscle. This was the background to Sherrington's own thinking and occupies the first three chapters. The story here brings out once more the strong opinions and antagonisms of the nineteenth century, as they developed out of the work of the seventeenth and eighteenth century. The final fourth chapter is an illuminating account of the development of the ideas which were summarized in the *Integrative Action of the Nervous System*, in 1906.

On reading this book one appreciates better the uncertainty about C.N.S. structure and function which prevailed at the time when Sherrington's work began. Although to-day such terms as the diastaltic nervous system are no longer in use, Prof. Liddell succeeds in reconstructing some of the old arguments, and in so doing he clarifies the meaning of these older terminologies and the points of view they reflected. The title of the book itself is an arresting reminder that an apparently simple act had slowly to be analysed piece by piece. The detailed account of how ideas on structure are dependent upon the tools available points its moral for the present day. The slow progress in the use of fixing and staining agents was ultimately the cause of many delays to progress. Stilling's accidental freezing of a piece of spinal cord exemplifies the occasional role of the casual in science. It is a measure of Prof. Liddell's patient scholarship that he can quote this as a re-discovery antedated by 200 years.

In this book of only 173 pages, some 316 names of the past are quoted and their contribution related. In an appendix there are set out the dates and some brief notes on the lives of some of the often forgotten personalities. This book is to be recommended to all students of the nervous system. Himself a student and friend of Sherrington, entering into physiology in the golden days of global spinal physiology, Prof. Liddell writes with knowledge and feeling and a dry humour which are not easily surpassed. C. B. B. DOWNMAN

An Introduction to Embryology. By B. I. BALINSKY. (Pp. 562; 291 figures: £2. 14*s*. 0*d*.) Philadelphia and London: W. B. Saunders Co. 1960.

Prof. Balinsky has set out to provide a book that will lay the shadow that he considers bedevils the teaching of embryology, namely the difficulty of co-ordinating the older data of descriptive embryology with the new discoveries resulting from the newer experimental outlook. He therefore presents embryology as a single science integrating the morphological with the physiological aspects. Embryology is interpreted in a very broad sense, presenting human as well as comparative developmental features. In addition to dealing with such topics as the origin of ova, fertilization, gastrulation, the determination of primary organ rudiments and organogenesis, it deals with embryonic adaptations including placentation, has a section on genetic control of the various processes, and considers the subjects of metamorphosis, regeneration and asexual reproduction.

Because of its scope the book is meant, and can only be considered, as a general introduction to Embryology. It is easy to read, and is not only ideally suited to students working for an honours degree in Anatomy or Zoology, but many sections could also be read with profit by medical students in general. All interested in the experimental approach to the medical and biological sciences will welcome this well-produced work.

T. W. GLENISTER

Missbildungen des Menschlichen Herzens: Entwicklungsgeschichte und Pathologie. By HEINZ BARTHEL. (Pp. vii + 237 and index; 215 illustrations; D.M. 188). Georg Thieme Verlag, Stuttgart. 1960.

The author is a senior surgeon at the Städt. Krankenhaus of Osterode (Harz). His book is in three parts. The first is an account of the parts played by growth and by haemodynamic factors in causing the formation of a divided heart with spirally disposed blood streams.

The second is an account of normal development. The third is a systematic account of cardiac malformations, including those of the aorta and pulmonary stems, interpreted in the light of the preceding sections.

Barthel's notion of the part played by haemodynamics is based upon a set of experiments upon models. The reviewer has repeated the basic experiments of the set and finds the author's thesis plausible. From his experiments the author concludes that the formation of endocardial cushions is due to pressure forces upon or within the cardiac wall, these pressures being due in part to kinkings produced by differential growth. The formation of cardiac septa, on the other hand, is due to haemodynamic suction (*Zugspannungen*) acting upon the interior of the cardiac tube, provided that the cushions have already been formed. With the exception to be noted later, the section on ontogeny is done thoroughly and unusually clearly. The section on malformations is equally thorough and clear, and is presented in a logical way. The reader's understanding of the whole text is helped enormously by the excellent illustrations made by Ingrid Schaumburg; she has used colour in a striking way for showing the several blood streams in a given heart.

The treatment of the pulmonary veins is unsatisfactory. Barthel plumps for their primary connexion with the left atrium without giving reasons, a procedure quite different from that which he adopts elsewhere when a choice has to be made. He does not cite the paper by Davies & MacConaill upon their unique case of bilocular heart (*J. Anat., Lond.*, **71**, 437); and his knowledge of the work of Chun Chang appears to be second-hand. Otherwise his account of the literature is good.

The book has a large page and clear type, and is elegantly printed. Its price, about £15, puts it beyond the private purse of most of us. But it does deserve a place in all libraries of both normal and pathological anatomy.

M. A. MACCONAILL

BOOKS RECEIVED

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HISTOCHEMICAL REACTIONS OF CELLULAR INCLUSIONS IN THE HUMAN NEURONE

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INTRODUCTION

In two earlier papers (D'Angelo, Issidorides & Shanklin, 1956; Shanklin, Issidorides & Nassar, 1957) we have reported our findings on neuronal inclusions such as neurosecretory material and lipofuscin pigment in the human hypothalamus, thalamus, motor cortex and cerebellum. Our results, obtained by histological and histochemical methods, indicate first, that neurosecretory material in the hypothalamus is of two varieties, and secondly, that many of the reactions that are generally considered specific for neurosecretory material are also shared by the lipofuscin of neurones outside the hypothalamus. This latter observation implies that the two most widely used histochemical methods for neurosecretion, viz. chrome-haematoxylin and aldehyde fuchsin, do not differentiate between neurosecretory material and lipofuscin, possibly because they possess chemical components in common that react similarly with the reagent used. It has been pointed out (Acher, 1958) that the Gomori-positive material in the hypothalamic secretory nuclei may represent a carrier substance. This substance may hold the antidiuretic and oxytocic hormones in a bound form until they reach their site of release into the blood stream. It is not unreasonable to suggest that lipofuscin may be a carrier for some other type of neuronal secretion. One very significant recent observation is that the sites of acid phosphatase and non-specific esterase activity in neurones coincide with the location of the pigment granules (Gedigk & Bontke, 1956). In the majority of the human neurones outside the hypothalamus there has been no convincing evidence of secretory activity in the accepted sense. In the human cerebellum, however (Shanklin *et al.* 1957), such evidence has been obtained. A precursor substance is found in the cytoplasm of the Purkinje cells in the form of very fine granules which are detected with great sensitivity by aldehyde fuchsin. These granules appear to develop later into the larger lipofuscin bodies found near the surface of the Purkinje cells, in the interstitium of the Purkinje cell zone and in the walls of nearby blood vessels.

The purpose of the present study is to investigate the lipofuscin in other areas of the human brain in order to obtain more evidence about its chemical nature, its morphological characteristics and its possible role as a neurohumour carrier. A preliminary report of these studies has been presented already (Shanklin & Issidorides, 1959).

MATERIALS AND METHODS

Our studies were based on sections from thirty-five human brains varying in age from the new-born to 84 years old. These brains were usually fixed in 10% formalin. Other fixatives were used in accordance with the particular histochemical method employed, namely dichromate-sublimate fixation (Elftman, 1957) for phospholipid demonstration with Sudan Black B, 1% trichloroacetic acid in 80% alcohol for the DDD method (Barrnett & Seligman, 1952), and Bouin's fixation for the chrome-haematoxylin method (Gomori, 1941). The remaining methods were applied on formalin-fixed tissue, namely, aldehyde fuchsin (Gabe, 1953), carbol-fuchsin gallocyanin (Einarson, 1953), periodic acid Schiff (McManus, 1948), luxol fast blue (LFB) PAS (Klüver & Barrera, 1953), silver diammine impregnation, with or without previous oxidation (Nassar, Issidorides & Shanklin, 1960), alloxan-Schiff (Yasuma & Ichikawa, 1953) counterstained with luxol fast blue (Shanklin & Issidorides, 1960) and alcian blue after performic acid oxidation (Adams & Sloper, 1955). The areas studied included parts of the medulla (i.e. inferior olive, nucleus of the hypoglossal nerve, arcuate nucleus), the supraoptic nucleus, the cerebellar cortex and the dentate nucleus. All the material used in this study was also examined unstained, mounted on quartz slides, with the fluorescence microscope.

OBSERVATIONS

The study of the distribution of lipofuscin inclusions in the brain is greatly facilitated by the application of aldehyde fuchsin. By this method we have obtained a high colour contrast between the granules and the surrounding cellular and interstitial tissues (Pl. 1, fig. 1; Pl. 2, figs. 19, 20). In the cells of the inferior olive aldehyde fuchsin (Pl. 1, fig. 1) reveals an intensely stained mass of lipofuscin pigment frequently occupying more than half the cell. The intensity of staining is such that the individual granules are not distinguishable, except occasionally at the periphery of the mass. When the same cells are stained by carbol fuchsin and luxol fast blue (Pl. 1, fig. 2), or by the alloxan-Schiff-LFB method (Pl. 1, fig. 3) the pigment mass has a different appearance. The mass still occupies the same volume of cytoplasm, but the individual granules are now very distinct. Each granule is intensely stained and is surrounded by a pale almost unstained layer (Pl. 1, figs. 2, 3). In a recent paper (Nassar *et al.* 1960) we have demonstrated that lipofuscin bodies consist of an inner core and an outer layer which may be selectively demonstrated by silver diammine after permanganate oxidation. This is the layer, we believe, that remains unstained in Pl. 1, figs. 2 and 3. However, when the olivary cells are stained with Sudan Black B after formalin fixation (Pl. 1, fig. 4) each pigment granule appears to be of a larger diameter and separated from its neighbours by irregular clear spaces. We conclude that this outer layer of the granule takes up Sudan Black B and that by this method we are observing the actual size of each lipofuscin body. When we stain the olivary cells with alcian blue after performic oxidation (Pl. 1, fig. 5) the appearance of the pigment mass is somewhat similar to that after aldehyde fuchsin. No distinct granules are seen, except at the periphery of the mass, and the staining is deep and intense. We interpret this result as indicating the presence of a third constituent in the pigment mass, probably a ground substance which permeates the

spaces between the lipofuscin bodies in that particular area of the cell. Our figures clearly illustrate that the aldehyde fuchsin and the alcian blue methods are the ones that demonstrate this ground substance. According to Pearse (1960) performic acid is the most suitable reagent for the oxidation of cystine. When combined with alcian blue staining this method has a high specificity, but a relatively low sensitivity and it therefore demonstrates only high concentrations of cystine in the tissues. On the other hand staining results with aldehyde fuchsin cannot be interpreted in a strict sense. However, among the substances for which aldehyde fuchsin has an affinity, after sulphuric permanganate oxidation, cystine is also included (Landing & Hall, 1956). It thus appears that the ground substance surrounding lipofuscin granules has a high content of cystine.

Our effort to determine the distribution of sulphydryl and disulphite groups has not yielded clear-cut results. For example, in the cells of the inferior olive and Purkinje cells of the cerebellum (Pl. 2, fig. 18) we have obtained after the Barrnett & Seligman method a deep brick-red homogeneous staining of the entire cell in trichloracetic fixed tissue. In formalin-fixed tissue, however, the same staining method has revealed darkly stained inclusions in the less intensely stained cytoplasm (Pl. 1, fig. 6). These inclusions do not have the exact distribution of the lipofuscin granules.

The pigment mass is also sharply differentiated by silver impregnation (Pl. 1, fig. 7; Pl. 2, figs. 15, 16). There are striking variations in the morphology of the pigment mass in each of these figures. The cells of the hypoglossal nucleus (Pl. 2, fig. 15) contain fine granules gathered in a compact mass. This arrangement is consistent throughout the cells of this nucleus and is similar to that in the giant pyramidal cells of the motor cortex (D'Angelo *et al.* 1956). In Pl. 2, fig. 15, there is again evidence of a brown-coloured ground substance between the lipofuscin granules. In the thalamus (Pl. 1, fig. 16), however, we consistently find fewer, but much larger round bodies localized on one side of the cell. A third variation in arrangement of the granules is that seen in the inferior olive (Pl. 1, fig. 7) cells. Thus each cell group in the brain appears to have its own characteristic 'lipofuscin pattern'. More specifically this pattern appears to be related to the morphological variety of the neurone (multipolar, fusiform, unipolar, etc.)

The relationship of the pigment to the cell organoids has been investigated by Einarson's gallocyanin method demonstrating ribonucleic acid containing proteins and by Elftman's method demonstrating phospholipids in the Golgi material. In the inferior olive cells (Pl. 1, fig. 8) Nissl material is found in a perinuclear position as intensely stained flakes, while the carbol fuchsin stained pigment area is entirely free of it. In the hypoglossal nucleus the pattern of distribution of these two substances is different. In the majority of cells (Pl. 1, fig. 12) we have found the typical Nissl pattern of multipolar neurones. Here the stained pigment granules are found between the coarse Nissl bodies. In some cells of the hypoglossal nucleus, however (Pl. 2, fig. 13), we have observed a very unusual relationship between Nissl material and pigment. The red pigment granules appear enclosed inside small blue crescents of Nissl substance. The association of the two substances is certainly close in this case and suggests more than a spatial coincidence of the two substances.

The relationship of lipofuscin granules to the Golgi bodies is more difficult to

determine since the Elftman method does not demonstrate both in the same section. Following this procedure we have observed in the cells of the inferior olive sharply defined Sudan Black B positive material occupying a small area of the cytoplasm adjacent to the nuclear membrane (Pl. 1, fig. 9). According to Elftman this method demonstrates the phospholipid part of the Golgi apparatus. His figures of intestinal epithelium (Elftman, 1957) support well this interpretation on morphological grounds. Our observations seem to lead to the same conclusion. First, Sudan Black B positive material is only found intracellularly near the nucleus; secondly, it appears as a network rather than as a granular mass, thirdly, it occupies a small area of the cytoplasm compared to the area occupied by lipofuscin.

After carbol fuchsin-LFB (Pl. 1, fig. 10), alloxan-Schiff-LFB (Pl. 1, fig. 11) and alcian blue (Pl. 2, fig. 14) the pigment in the cells of the hypoglossal nucleus has the distribution of pyramidal-shaped masses on one or two sides of the nucleus.

In all these studies we have been particularly interested in the results obtained by the alloxan-Schiff method which give a strong indication of the presence of protein in the pigment. We have sought to compare the reactivity of the neurosecretory substance in the cells of the supraoptic nucleus (Pl. 2, fig. 17) with that of the lipofuscin. Both give a positive reaction of the same intensity with the alloxan Schiff method. In Pl. 2, fig. 17, the neurosecretory material at the periphery of the neurones appears as agglomerations of large red globules and has the same distribution as the material stained by chrome haematoxylin. The same red globules are also found in the processes of the supraoptic neurones. These two observations indicate that the alloxan-Schiff method does indeed demonstrate a protein component of the neurosecretory substance in the hypothalamus, and furthermore that the lipofuscin contains a protein component in a similar concentration. None of the methods applied thus far demonstrate the second variety of neurosecretion, which is aldehyde-fuchsin positive, found in a central position inside the supraoptic neurones and in their processes (Shanklin *et al.* 1957). We are currently engaged in investigating this problem further.

Finally, our results on the cerebellum need special attention. As illustrated in our material aldehyde fuchsin demonstrates very fine granules in the Purkinje cells and larger bodies of identical staining in the interstitium of the Purkinje cell zone (Pl. 2, fig. 19) and lipofuscin in the cells of the dentate nucleus (Pl. 2, fig. 20). We have found also intensely stained bodies in the walls of the nearby blood vessels of the Purkinje cell zone. Out of this group of bodies, alloxan-Schiff, on the other hand, demonstrates only the vascular deposits (Pl. 2, fig. 21) and the lipofuscin in the dentate nucleus (Pl. 2, fig. 22). This indicates that the composition of these bodies in the cerebellum is not uniform and that they probably incorporate other substances as they develop from the fine precursor granules in the Purkinje cells to the larger deposits in the blood vessels. Incidentally our figures also illustrate the two types of neurones in the dentate nucleus as described by Höpker (1951). These are large multipolar ones (Pl. 2, fig. 20) and a smaller fusiform variety (Pl. 2, fig. 22).

Fluorescence studies show that in all our sections the lipofuscin inclusions have a strongly yellowish fluorescence. This is also true in the area of the inferior olive and in the interstitium of the Purkinje cell zone.

DISCUSSION

Our investigation of the morphological characteristics of the lipofuscin granules in the human inferior olive cells reveals that each granule is a complex of lipid and protein in successive concentric layers. This structural interpretation is supported by birefringence studies (Scharf, 1952) which indicate the presence of lipid molecules orientated in a radial direction as usually seen in liproprotein membranes. Acid-fast material and protein as demonstrated by carbol-fuchsin and alloxan-Schiff (Yasuma & Ichikawa, 1953), respectively, constitutes the core of the granules. A positive reaction with Sudan Black B on formalin-fixed sections, according to Pearse (1960), signifies 'formol-fixed' lipid which is usually phosphatide or cerebroside. This positive reaction occurs on the outer layer of the granules. The presence of these substances in lipofuscin has also been established by chemical analysis (Casselman & Baker, 1955). Additionally we have demonstrated a high concentration of cystine on the surface and between the granules in the pigment mass. The intensity of the alcian blue colour reaction after performic acid oxidation is a measure, according to Pearse (1960), of the concentration of cystine. This substance has been demonstrated thus far in high concentrations only in the neurones of the hypothalamic nuclei (Adams & Sloper, 1956) while it is apparently absent from neurones in other nuclei. Our results clearly indicate that this is not the case in the cells of the inferior olive and hypoglossal nucleus. It therefore appears that a substance rich in cystine is associated with the lipoprotein in the lipofuscin granules. As mentioned before, the positive reaction with aldehyde fuchsin is not indicative of a particular group or substance, although cystine-containing substances have been included among others reacting with aldehyde fuchsin (Landing & Hall, 1956).

Our earlier work (Shanklin *et al.* 1957) has revealed that in the Purkinje cells of the cerebellum, masses of pigment appear in the areas occupied by the Golgi network. This association would indicate that the formation of pigment may be a result of synthetic cellular activity. The formation of melanin by the Golgi body in human malignant melanomas has been demonstrated recently with the electron microscope. (Wellings & Siegel, 1959). A complete parallel cannot be drawn for lipofuscin; the fact, however, that both melanin and lipofuscin are neuronal pigments allows us to consider the possibility that either the Golgi bodies (see also Gatenby & Moussa, 1951), or other cellular organoids are active in its formation. In this present study it appears that there is an association of lipofuscin and Nissl material, at least in the cells of the hypoglossal nucleus. This corroborates the findings of Hydén & Lindström (1950), who have demonstrated that there is an inverse relationship between the amount of pigment present in the neurone and Nissl substance. Actually the pigment increases while the Nissl material decreases as the cells develop (Pope & Hess, 1957). Scharrer, Palay & Nilges (1945) have also found that neurosecretory material in the hypothalamus appears to originate within the Nissl substance. The fact that there is no complete agreement as to where the pigment originates is further evident if we consider the work of Hess (1955) on guinea-pig neurones with the electron microscope. He has found that pigment granules are formed through gradual changes of mitochondrial outer membranes. The concept that lipofuscin is a waste product of the neurone no longer seems to be justified.

Recent experimental work (Sulkin & Srivanij, 1960) has shown that extrinsic factors are related to the formation or increase of pigment in the nerve cells. Earlier Einarson (1953) had also shown that a dietary factor such as lack of vitamin E causes accumulation of pigment in the neurones. Gedigk & Bontke (1956) suggest that a physiological role should be assigned to the pigment, since they have demonstrated by chemical analysis acid phosphatase and non-specific esterase having the exact distribution of the lipofuscin granules.

Comparing results on the medulla with those already described in the cerebellum we may draw two conclusions. First, in the cerebellum we have evidence of a secretory cycle, since we find Purkinje cells in different stages of activity with respect to pigment formation, accumulation and probably release into the surrounding interstitium. On the other hand in the medulla no such cycle is noticeable, the staining and histochemical reactions, however, have definite similarities to those of the hypothalamic neurosecretory cells.

We conclude then that in both these areas there are cells possessing characteristics of secretory activity but that their mechanism of secretion, if such is the case, is not the same, and also that this mechanism differs from that of hypothalamic neurosecretion. In the cerebellum we have large quantities of lipofuscin interstitial material, sharply localized in the Purkinje cell zone and dentate nucleus, while in the medulla similar material is localized in the interstitium of the inferior olive zone. The common denominator of these three areas is that they are very rich in synapses. It is impossible to tell at this stage whether any significance should be attached to this. However, we can conclude that regardless of its mechanism of origin this extracellular lipofuscin accumulates in the neighbouring synaptic fields.

Another point that should be made is that the more we evaluate the staining and histochemical reactions of these neuronal inclusions and interneuronal material, the more evident it becomes that one set of properties cannot be ascribed to all of them. In all the areas studied thus far we have found that the two common properties are yellow-white fluorescence in ultra-violet light and intense reactivity with aldehyde fuchsin. Other staining properties vary. Two possible interpretations may be suggested. If these bodies perform one definite function in the human brain their fluorescence and aldehyde fuchsin positivity should be the clues for further investigations as to their mode of action. If, on the other hand, a variety of functions is to be assumed, depending on the areas studied, then we should probably view the properties of fluorescence and aldehyde fuchsin positivity as belonging to a carrier substance which becomes associated with a variety of active substances as the need arises and depending on the particular brain area.

An interesting example of neurosecretion outside the hypothalamus is found in the eel. Holmgren (1959) has described in this animal neurosecretory cells in the spinal cord with axons that transport the material into the urophysis spinalis.

SUMMARY

1. The distribution and histochemical reactions of lipofuscin inclusions in the human medulla, cerebellum, thalamus and hypothalamus have been investigated by a variety of histochemical methods.

2. The size, shape and distribution of the lipofuscin inclusions are similar in all cells of a given nucleus (cell group). These features vary widely, however, in the different cell groups studied. Each cell group in the brain may be said to have a characteristic 'lipofuscin pattern'.

3. In the inferior olive each lipofuscin granule is a complex of lipid and protein in successive concentric layers. There is some evidence that phosphatides, or cerebrosides, are present in the granules. Cystine-containing proteins also appear to be present in the ground substance surrounding the lipofuscin granules.

4. The intensity of staining of lipofuscin granules after the alloxan-Schiff method for proteins is similar to that of hypothalamic neurosecretory substance.

5. Some evidence suggests that the lipofuscin granules, at least in the cells of the hypoglossal nucleus, originate among the Nissl bodies.

6. Morphological and histochemical evidence suggests that the Purkinje cells and inferior olive cells may have a secretory mechanism for the formation of lipofuscin. Also abundant extracellular lipofuscin granules are found widely dispersed in the synaptic fields surrounding these particular cell groups.

7. Hypotheses on the possible role of lipofuscin in the neurons are discussed.

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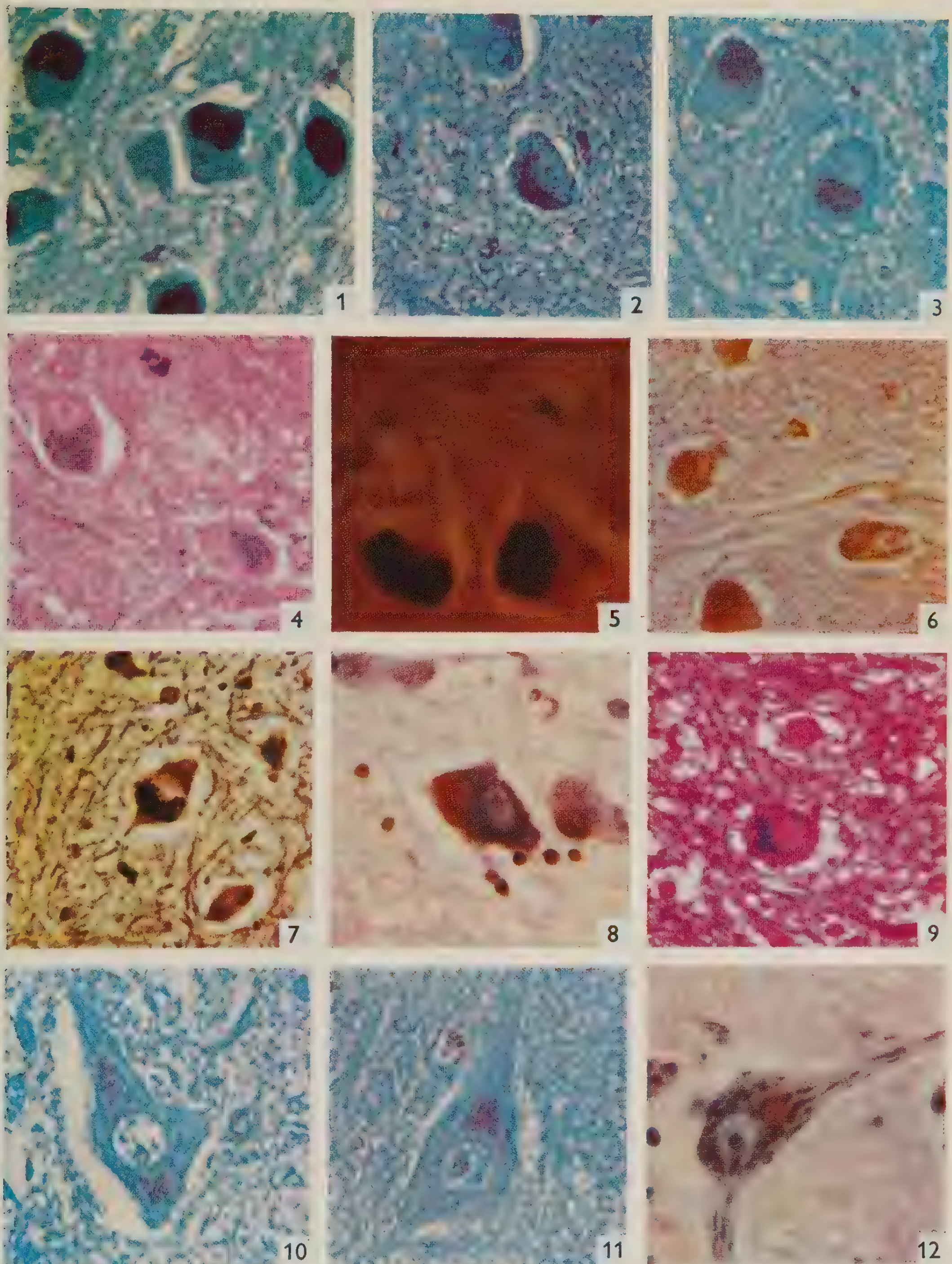
EXPLANATION OF PLATES

Plate 1

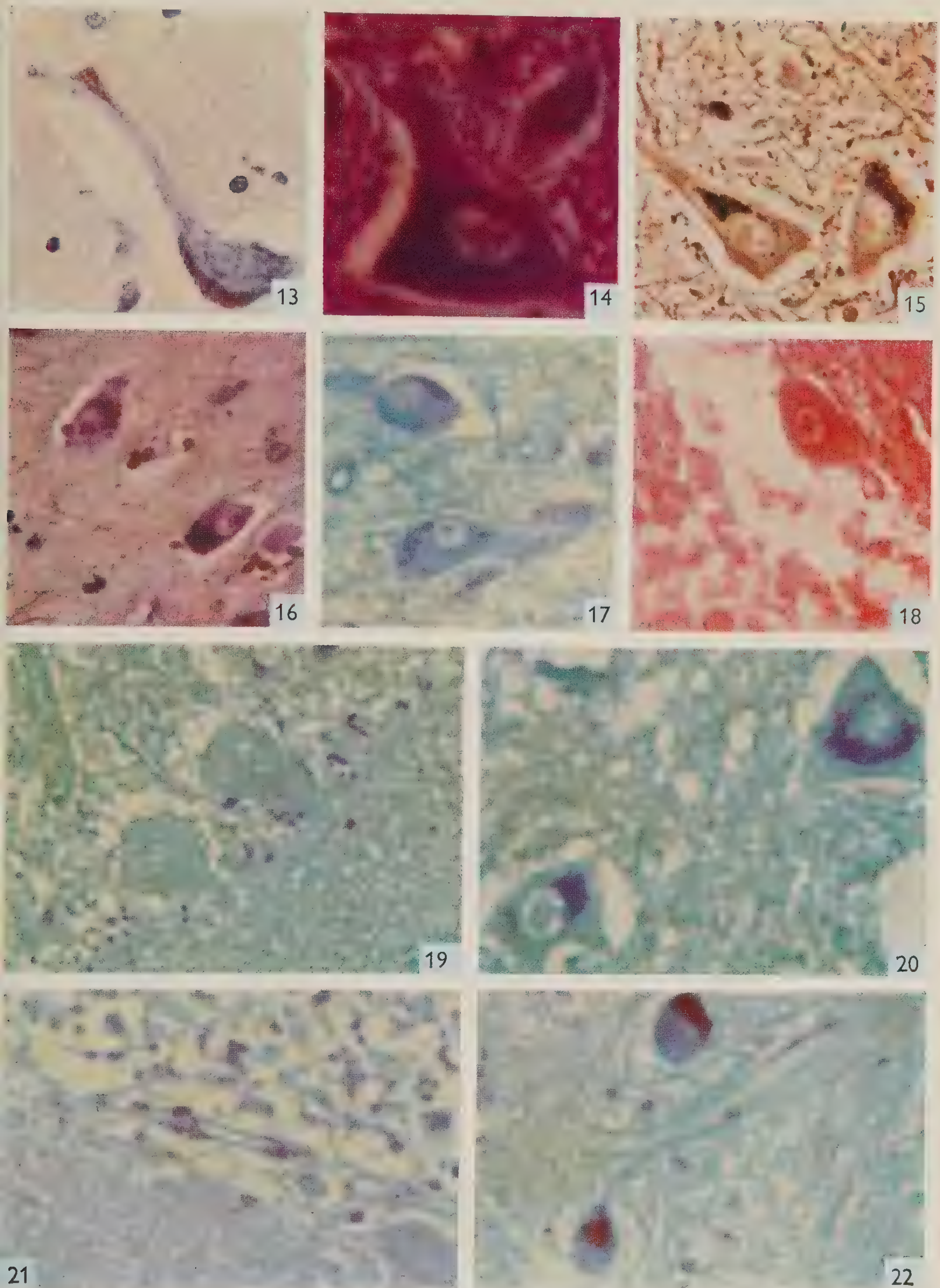
All figures are photomicrographs of human brains between the ages of 40 and 75 years. All are from formalin fixed material, except where noted otherwise.

Fig. 1. Cells from inferior olive stained with aldehyde fuchsin. ×300.
Fig. 2. Cells from inferior olive stained with carbol fuchsin and luxol fast blue. ×300.
Fig. 3. Cells from inferior olive stained by alloxan-Schiff method and luxol fast blue. ×300.
Fig. 4. Cells from inferior olive stained with Sudan Black B. ×300.
Fig. 5. Cells from inferior olive stained with alcian blue and counterstained with erythrosin × 500.



ISSIDORIDES AND SHANKLIN—HISTOCHEMICAL REACTIONS OF CELLULAR INCLUSIONS IN THE HUMAN NEURONE

(*Facing p.* 158)



ISSIDORIDES AND SHANKLIN—HISTOCHEMICAL REACTIONS OF CELLULAR INCLUSIONS IN THE HUMAN NEURONE

Fig. 6. Cells from inferior olive stained by the Barrnett–Seligman method. Fixation in 1% trichloracetic acid in 80% alcohol. ×300.

Fig. 7. Cells from inferior olive following silver impregnation. ×300.

Fig. 8. Cells from inferior olive stained by the Einarson gallocyanin-chromalum method. ×300.

Fig. 9. Cells from inferior olive stained by Sudan Black B. Fixation by Elftman dichromate-sublimate method. ×300.

Fig. 10. Cells from hypoglossal nucleus stained by carbol fuchsin and luxol fast blue. ×300.

Fig. 11. Cells from hypoglossal nucleus stained by the alloxan-Schiff method and luxol fast blue. ×300.

Fig. 12. Cells from the hypoglossal nucleus stained by the Einarson gallocyanin-chromalum method. ×300.

PLATE 2

Fig. 13. Cells from hypoglossal nucleus stained by the Einarson gallocyanin-chromalum method. ×300.

Fig. 14. Cells from hypoglossal nucleus stained with alcian blue and counterstained with erythrosin. ×500.

Fig. 15. Cells from hypoglossal nucleus following silver impregnation. ×300.

Fig. 16. Cells from thalamus following silver impregnation and staining by the alloxan-Schiff method. ×300.

Fig. 17. Cells from supraoptic nucleus stained by the alloxan-Schiff method. ×300.

Fig. 18. Purkinje cell zone of the cerebellum stained by the Barrnett–Seligman method. Fixation in 1% trichloracetic acid 80% alcohol. ×300.

Fig. 19. Purkinje cell zone of cerebellum stained with aldehyde fuchsin. ×300.

Fig. 20. Cells from dentate nucleus stained with aldehyde fuchsin. ×500.

Fig. 21. Purkinje cell zone of cerebellum stained by the alloxan-Schiff method. ×300.

Fig. 22. Cells from dentate nucleus stained by the alloxan-Schiff method. ×300.

A QUALITATIVE AND QUANTITATIVE STUDY OF THE MYENTERIC PLEXUS OF THE SMALL INTESTINE OF THE CAT

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INTRODUCTION

Dogiel (1896, 1899) was the first to observe that the nerve cells of the sympathetic ganglia and the ganglia of the gut wall could be divided into two or three types according to their staining reactions and morphology. His findings are still substantially valid and have been confirmed by recent neuro-histological investigations (Yamauchi, 1958; Wada, 1958; Rintoul, 1959).

The purpose of the present investigation was to study the distribution of the different types of nerve cells on a quantitative basis and to explore their histochemical reactions in respect of cholinesterase activity.

The cat was chosen for this work because it is frequently used in experimental research on the alimentary tract.

MATERIAL AND METHODS

The material consisted of the gastro-intestinal tract and sympathetic ganglia of twenty-seven adult cats killed by intraperitoneal injection of nembutal.

For neuro-histological work the specimens were fixed in 10% neutral formalin for at least 3 days. Frozen sections 20 μ in thickness, of duodenum, jejunum and stomach were cut parallel and perpendicular to the peritoneal surface. Care was taken to flatten out the specimens during freezing so that all the myenteric plexus in an area of approximately 100 mm.^{2} was contained in about fifteen sections. The sections were usually stained by a simplified Bielschowsky–Gros silver impregnation method (Cauna, 1959). Some sections were treated with ferric chloride (Dixon, 1958) or stained with methylene blue or other routine stains.

To study the distribution and frequency of the nerve cells in the myenteric plexus, serial sections cut parallel to the peritoneal surface of the gut wall were stained with silver and photographed. The nerve cells in the sections were identified under the microscope and marked on the photographs, which were then carefully superimposed. In this way the whole pattern of the nerve cells was mapped out in an area of 6·75 mm.^{2} of duodenum of one cat and in 93·3 mm.^{2} of upper jejunum of another cat.

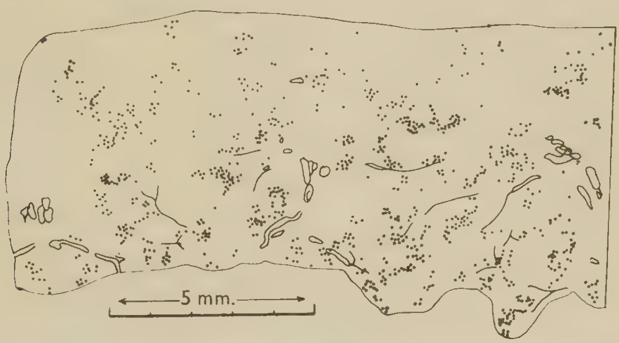
The specimens intended for histochemical work were fixed in 10% neutral formalin for three to 6 hr. and then frozen sections 20–30 μ in thickness were cut. These were collected in distilled water, washed for 30 min. and then incubated with a substrate from 1 to 17 hr. at 37° C. using the histochemical technique of Koelle

(1951) as modified by Snell (1959). The pH of the substrates was lowered to 5·3 in order to reduce diffusion artifacts. Eserine controls were used with both substrates, and these always showed a negative reaction.

OBSERVATIONS

Neuro-histological findings

In the upper jejunum, the myenteric plexus was found to be situated 50–150 μ from the peritoneal surface of the gut. The variation in depth was due to the variation in thickness of the longitudinal muscle coat (Pl. 1, fig. 1). The nerve cells of the plexus were found to be arranged in clusters of variable size and shape. In the antimesenteric part of the plexus there were fewer cells than in the mesenteric part, and the clusters tended to be smaller and some single nerve cells were seen (Text-fig. 1).



Text-fig. 1. The distribution of the deeply staining nerve cells (solid dots) of the myenteric plexus of the upper jejunum. The tracing is based on fourteen serial frozen sections 20 μ in thickness stained with silver, and the outlined area represents 93·3 mm.^{2} of the gut wall extending from the mesenteric border (lower part of the figure) to the antimesenteric border (top part of the figure). The line tracings in the mesenteric half of the figure indicate the position of the large blood vessels. The total number of deeply staining nerve cells is 982 or 10·5 cells mm.^{2} Adult cat, $\times$ 5·3.

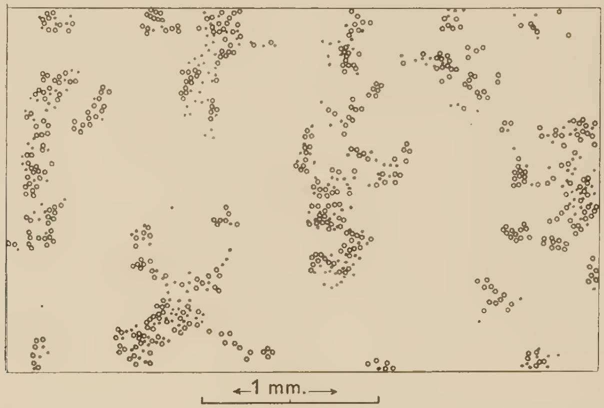
Large nerve bundles were frequently found with large blood vessels but the ganglia of the plexus did not show any definite relation to the vessels.

In the duodenum the plexus contained more nerve cells in rather larger clusters than in the jejunum (Pl. 1, fig. 2, and Text-fig. 2).

Both in jejunum and duodenum, the nerve cells of the myenteric plexus showed a variable affinity for silver. Some cells stained very heavily, others stained lightly and a large number remained almost unstained; these could only be positively identified by phase contrast microscopy. (Pl. 1, figs. 3, 4). In the jejunum the nuclei of the nerve cells usually stained lightly and the deeper staining nucleoli were easily seen (Pl. 1, fig. 3), whereas in the duodenum the nuclei stained heavily and the nucleoli were obscured (Pl. 1, fig. 4). In both parts of the gut the nerve cells of a ganglion showed a wide variety of shapes ranging from spindle-shaped bipolar neurons to symmetrical or asymmetrical multipolar cells (Pl. 1, figs. 2–4).

In the upper jejunum a study was made of the frequency and distribution of the deeply staining nerve cells of the myenteric plexus in an area of 93·3 mm.^{2}. This

area extended from the mesenteric border of the gut to the anti-mesenteric border. The map used for this study is shown in Text-figure 1. The larger blood vessels were also traced and were found to be concentrated in the mesenteric half of the specimen (lower half of figure). The average frequency of the deeply staining nerve cells was found to be 10·5 cells/mm.^{2} but it was also found that their distribution was not uniform there being many more dark cells in the mesenteric zone of the gut than in the antimesenteric zone. Even in the antimesenteric zone, however, any chosen point was less than 1 mm. away from a nerve cell or group of nerve cells. The lightly staining cells were not mapped out in jejunum but were counted under the microscope using the same technique as for a differential white cell count.



Text-fig. 2. The distribution of the deeply staining nerve cells (solid dots) and the lightly staining nerve cells (circles) of the myenteric plexus of the duodenum. The tracing is based on five serial frozen sections 20 μ in thickness stained with silver, and the area represents 6·75 mm.^{2} of the gut wall near the mesenteric border. The total number of deeply staining nerve cells is 287 or 42·5 mm.^{2} and of the lightly staining cells 535 or 79·2 mm.^{2} Adult cat, $\times$323.

It was found that of 836 cells counted, 287 were dark cells and 549 were lightly staining resulting in the ratio of dark cells to light cells of 1:1·9. Using this ratio the average frequency of dark cells and light cells together in the myenteric plexus of the jejunum would be 30·7 cells/mm.^{2}, or considering the mesenteric and antimesenteric zones separately the respective figures would be 37·7 and 17·7.

In the duodenum both the deeply staining and the lightly staining nerve cells of the myenteric plexus were mapped out in an area of 6·77 mm.^{2} taken from the mesenteric region (Text-fig. 2). The average frequency of nerve cells was found to be 122 cells/mm.^{2} and the ratio of dark cells to light cells was 1:1·9 as calculated for the jejunum.

Cholinesterase reaction

After incubating sections of gut with acetyl substrate, cholinesterase positive and negative nerve cells were found in the myenteric plexus. About equal numbers of nerve cells gave negative or strongly positive reactions but a small number of the cells showed a weak positive reaction. In the cholinesterase positive cells the black copper sulphide deposit was evenly distributed throughout the cytoplasm but

was not present in the nuclei. The tissues surrounding the nerve cells gave a strong positive reaction so that the cholinesterase negative cells were clearly delineated whereas the outlines of the positive cells were difficult to see (Pl. 2, fig. 5). When butyryl substrate was used all the nerve cells in the myenteric plexus gave a negative reaction but again the surrounding tissues were strongly positive (Pl. 2, fig. 6). It was not possible to determine the exact localization of cholinesterase activity in the tissues around the nerve cells. The distribution of the deposit was remarkably uniform even after short periods in incubation although there were some lighter spots which appeared to be the nuclei of the supporting cells. The appearance suggested that the enzyme was contained within the cytoplasm of the supporting cells, the boundaries of which could not be seen. Nerve fibres related to the nerve cells and contained within the cell membranes of the supporting cells (Richardson, 1958; Taxi, 1958) may also be cholinesterase positive but these could not be identified with the optical microscope. The interstitial cells of Cajal did not give a positive reaction with either substrate. For the sake of comparison sections of sympathetic ganglia of the same animals, together with the specimens of the gut, were incubated under identical conditions. In the superior cervical ganglion the vast majority of the nerve cells were cholinesterase negative after incubation with acetyl substrate, but occasional solitary cells gave a strong positive reaction and a few cells gave a reaction of intermediate intensity (Pl. 2, fig. 7). In the stellate ganglion the appearances were similar but the proportion of cholinesterase positive cells was greater than in the superior cervical ganglion. In both ganglia the tissues surrounding the nerve cells gave a positive reaction, but this was less intense than in the myenteric plexus so that the cholinesterase positive cells were very conspicuous (Pl. 2, cf. fig. 5 with fig. 7). When the sections were incubated with butyryl substrate all nerve cells of the sympathetic ganglia gave a negative cholinesterase reaction but the surrounding tissues were strongly positive—a picture very similar to that of the myenteric plexus after incubation with the same substrate (Pl. 2, cf. fig. 6 with fig. 8).

After prolonged incubation with acetyl or butyryl substrates certain tissues which are not part of the nervous system of the gut also gave a positive cholinesterase reaction which varied in degree. The muscle cells of the longitudinal coat and the muscularis mucosae were positive (Pl. 2, figs. 9, 10), while the muscle cells of the circular muscle coat gave a negative reaction. The capillaries in the circular muscle coat gave a definite positive cholinesterase reaction. Some cells of the intestinal glands were also positive (Pl. 2, figs. 9, 10).

DISCUSSION AND CONCLUSIONS

The distribution of the nerve cells in the myenteric plexus is of interest not only to the anatomist but also to the physiologist working with micro-electrodes. It may be of value in the planning of electro-physiological experiments to know that the nerve cells of the plexus are situated only 50–150 μ from the peritoneal surface of the gut and that they are more numerous in the mesenteric zone.

Counts of the nerve cells of the myenteric plexus have been carried out by a number of investigators on several different species of animals, but differential counts of deeply and lightly staining nerve cells are not reported in the literature

available to the authors. Only our total cell counts, therefore, can be used to compare the present findings with those of earlier workers. In the mesenteric zone of the duodenum the average frequency of deeply and lightly staining nerve cells was 121·7 cells/mm.^{2} In the upper jejunum where the whole width of the gut wall was used the average figure was 30·7 nerve cells/mm.^{2} If the mesenteric and antimesenteric zones of the jejunum are considered separately the respective values were 37·7 and 17·7 nerve cells/mm.^{2} To compare these figures with other published work they have to be expressed as the number of cells per square centimetre. Table 1 shows the comparison of the collected figures.

Table 1. *Frequency of nerve cells in the myenteric plexus of the small intestine*

Author	Animal	Gut	Nerve cells/cm.^{2}
Irwin, 1931	Guinea-pig	Mid-duodenum	10,000
		Small intestine including duodenum	7,500
Matsuo, 1934	Guinea-pig	Duodenum	9,100–9,800
		Ileum	7,200
Ohkubo, 1936*a*	Guinea-pig	Duodenum	6,700
		Ileum	5,300
Ohkubo, 1936*b*	Monkey	Duodenum	2,700
		Jejunum	2,700
		Ileum	2,400
Sauer & Rumble, 1946	Cat	Duodenum	49,081
		Ileum	15,411
Tafuri, 1957	Guinea-pig	Ileum	14,200
Tafuri & de Almeida Campos, 1958	Mouse	Ileum	29,600
Present investigation, 1960	Cat	Duodenum, mesenteric zone	12,170
		Jejunum: Average	3,070
		Mesenteric zone	3,770
		Antimesenteric	1,770

The figures in the last column of Table 1 show that most workers agree that the nerve cell frequency in the myenteric plexus is higher in the duodenum than it is in the more distal part of the small intestine. The actual figures, however, vary greatly even in a single species and it may be that the results are influenced by various technical factors. Probably the most important of these is the extreme difficulty in identifying all the nerve cells positively because of their variable staining reactions. Other important factors which could produce genuine differences in results are the situation of the area of gut chosen for the count in relation to the mesenteric border and the part of the gut examined—duodenum, jejunum or ileum. Precise information on these two points was not always available in the quoted publications. The shrinkage of tissues during fixation was not found to be a significant factor in this work as was shown by the following experiment. Pieces of small intestine were taken from a cat at the time of death and permanent marks were made from which accurate measurements were taken in longitudinal and transverse directions. The specimens were then fixed in formalin some pinned out flat and others not. Later measurements showed that there had been practically no shrinkage of the tissues of any of the specimens.

Differential affinity of nerve cells for silver stains has been frequently registered by a number of investigators and the present study confirms such findings. The question arises as to whether these staining differences can be used as a safe basis for classifying nerve cells into two or three types as suggested by Dogiel (1896, 1899). It is well known that the results obtained by neuro-histological stains depend upon various factors some of which are difficult to control. Apart from this consideration, however, it may be that different staining reactions signify temporary changes in neurones depending upon their state of activity.

The histo-chemical findings in respect of cholinesterase activity in the myenteric plexus may throw some light on these two questions. It was found that with acetyl substrate roughly equal numbers of cells gave negative or strongly positive reactions and a smaller number showed an intermediate degree of positive reaction. It seems possible, therefore, that the different staining characteristics of the nerve cells revealed by neuro-histological techniques may be related to their enzyme content. To investigate this possibility, some sections incubated with acetyl substrate were subsequently stained with silver. The results were not conclusive because the silver impregnation was not entirely satisfactory but those cells which did stain were all cholinesterase negative; this provides some indication that the argyrophil neurones may correspond with the cholinesterase negative nerve cells; and the neurones which stain lightly or remain unstained—with the cholinesterase positive cells. This, however, has to be confirmed by further investigations.

The cholinesterase studies provide some support for Dogiel's suggestion that visceral ganglia contain two or three types of nerve cells. The observed variations in the enzyme content of the nerve cells must represent a difference of cell type and not merely a difference in their functional state because histochemical experiments carried out on a number of animals under varying conditions always give the same general results. It is also known that cholinesterase is not immediately lost from the motor end plates after sectioning the motor nerve, but disappears gradually over a period of several weeks (Bergner, 1957; Clodius, 1958). In addition, experiments in our own laboratory by Thakar Naik (personal communication) show that sympathetic ganglia can be stored at 4° C. or in a frozen condition for several days without any detectable change in the differential cholinesterase reactions in the nerve cells or in the surrounding tissues.

The fact that, certain non-nervous tissues of the wall of the intestine give a positive cholinesterase reaction of varying degree requires further consideration. The cholinesterase content of the longitudinal muscle layer and the muscularis mucosae of the small intestine of the cat is high in comparison with that of the circular muscle layer. The capillaries of the circular muscle coat contain more cholinesterase than the muscle cells surrounding them and certain cells of the intestinal glands also give a positive reaction. The significance of these findings is a matter of speculation but in our opinion the enzyme may play a role in the neuro-effector mechanism. Neuro-histological studies have shown that in areas of autonomic nerve supply there is not an individual nerve terminal for each unstriped muscle fibre and each glandular cell. It has been suggested by a substantial number of investigators that a syncytium of interstitial cells exists which forms an intermediate system between the terminal nerve fibres and the tissues they supply (Meyling, 1954; Jabonero, 1954; Kuntz &

Napolitano, 1956; Knoche, 1958). Our studies showed that the interstitial cells of the myenteric plexus of the intestine of the cat had a cholinesterase negative reaction. A similar observation was made by Coupland & Holmes (1958) on the same cells in the rabbit. These negative cholinesterase findings do not provide support for the theory of nervous transmission by means of the interstitial cells but agree with the observations of Richardson (1958). Using the electron microscope he studied the spacial distribution of the interstitial cells and in particular their relationship with nerve fibres on the one hand and muscle cells on the other. He concluded that the interstitial cells could not form the final link between the nerves and the muscle cells.

If the interstitial cells do not form the neuro-effector mechanism of the peripheral autonomic nervous system an alternative possibility may be considered. As no discrete endings have been found in autonomic nerves it seems probable that the whole length of the terminal segment of the autonomic fibre may constitute a zone of transmission (Alberti & Cauna, 1960). The cholinesterase found in tissues supplied by these nerves may play a part in the transmission and propagation of the nervous impulse just as it does in striated muscle fibres (Nachmansohn, 1959).

The chemistry of nervous transmission is not yet understood even at synaptic junctions (Paton, 1958). In Meissner's corpuscles where cholinesterase is localized at the pre-synaptic membrane of the laminar cells the enzyme may be associated with permeability changes of that membrane (Cauna, 1960). On the other hand cholinesterase is found in a variety of tissues not associated with nervous action (erythrocytes, placenta) and it is not inevitably concerned with the hydrolysis of acetylcholine (Gerbtzoff, 1959).

One practical application which may emerge from the speculations about the probable role of cholinesterase in non-nervous tissues is concerned with the cause of ulceration in the alimentary tract. A study of the distribution of cholinesterase activity in non-nervous tissues of the normal gastro-intestinal tract and in the area of the ulcer may cast some light on this important problem. Investigations along these lines are being carried out in our laboratory at the present time on human gastric ulcer.

SUMMARY

Neuro-histological and histochemical studies have been carried out on the myenteric plexus of the duodenum and jejunum of twenty seven adult cats using a simplified Bielschowsky–Gros silver method and a modified Koelle's histochemical technique for cholinesterase reaction.

It was found that the myenteric plexus was situated 50–150μ deep to the peritoneal surface. The number of nerve cells per square centimetre was found to be 12,170 in the duodenum and 3700 in the jejunum. In the jejunum, the mesenteric zone of the plexus contains many more nerve cells than the antimesenteric zone. The ratio between deeply and lightly staining nerve cells was found to be 1:1·9 in both duodenum and jejunum.

After sections of gut had been incubated with acetyl substrate some of the nerve cells of the plexus gave a positive and others a negative cholinesterase reaction, but with butyryl substrate they all gave a negative reaction. The tissues surrounding

the nerve cells gave a positive reaction with both substrates and the interstitial cells of Cajal were negative.

The longitudinal muscle coat, the muscularis mucosae the capillaries of the circular muscle coat and some cells of the intestinal glands gave a positive cholinesterase reaction after prolonged incubation.

The significance of the findings is discussed.

We gratefully acknowledge the technical assistance of Miss Jill S. Hocknell. Our thanks are also due to Mr C. J. Duncan and the staff of the Photography Department for their aid and co-operation with the photographic work and to Miss Dorothy Mustart for preparing the drawings of Text-figures 1 and 2. One of the authors (N.C.) acknowledges an equipment grant from the Royal Society.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Field of a transverse section of the upper jejunum showing the myenteric plexus between the longitudinal (L) and circular (C) muscle coats. Some deeply staining nerve cells (arrows) and bundles of nerve fibres can be recognized in the plexus. Adult cat. Frozen section, $20\,\mu$. Simplified Bielschowsky–Gros silver impregnation. $\times 167$.

Fig. 2. Field of horizontal section of the duodenum showing the myenteric plexus between the longitudinal (L) and circular (C) muscle coats. The deeply staining nerve cells occur in a variety of shapes. Some lightly staining nerve cells are indicated by arrows. Adult cat. Frozen section, $20\,\mu$. Simplified Bielschowsky–Gros silver impregnation. $\times 167$.

Fig. 3. Field of the myenteric plexus of the upper jejunum showing a deeply staining spindle-shaped nerve cell with lightly staining nucleus and two lightly staining nerve cells recognized by their vesicular nuclei and deeply staining nucleoli (arrows). Adult cat. Frozen section, $20\,\mu$. Simplified Bielschowsky–Gros silver impregnation. $\times 500$.

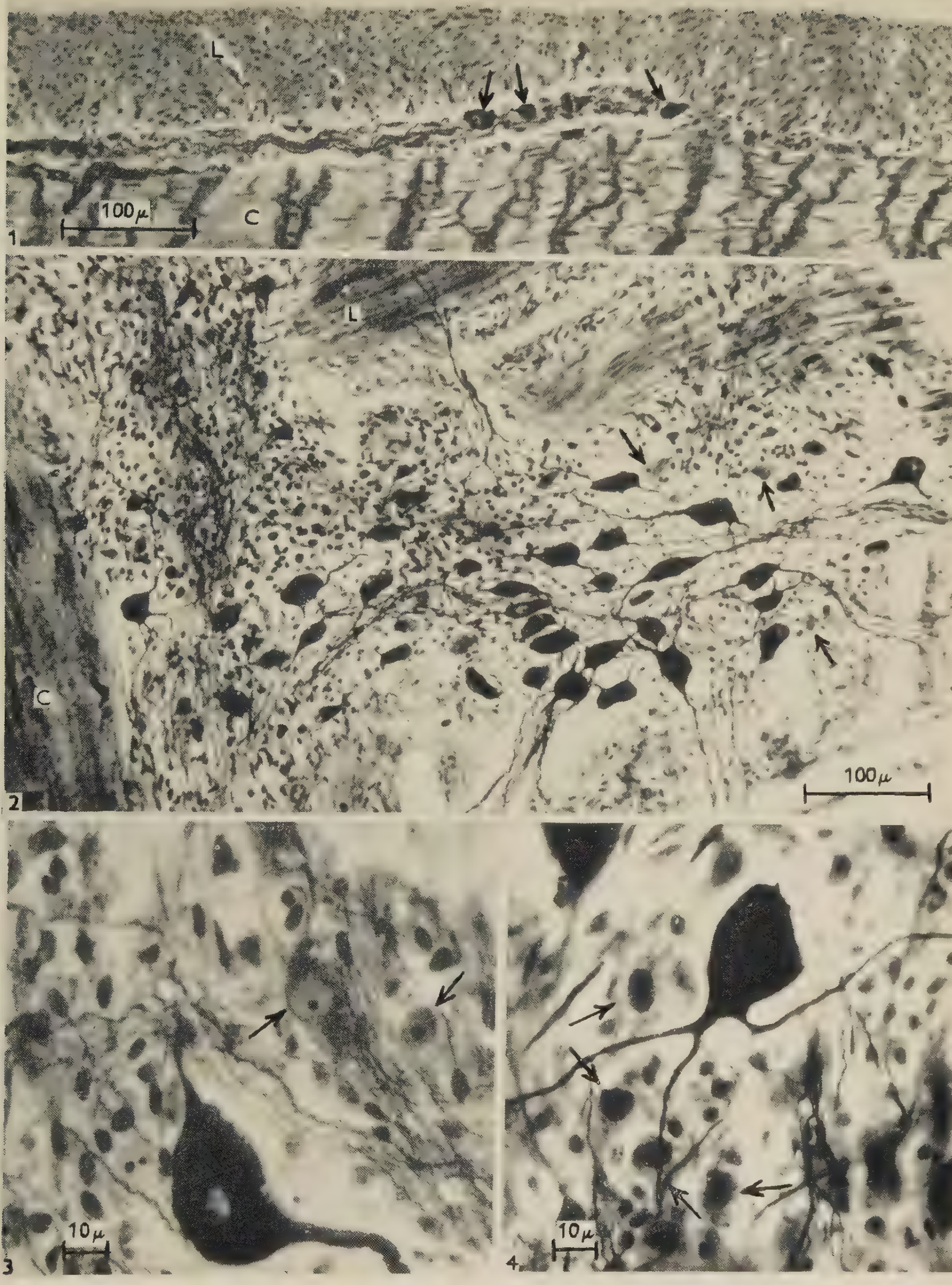
Fig. 4. Field of the myenteric plexus of the duodenum showing a deeply staining asymmetrical multipolar nerve cell and a number of lightly staining nerve cells recognized by their dark nuclei (arrows). Adult cat. Frozen section, $40\,\mu$. Simplified Bielschowsky–Gros silver impregnation. $\times 500$.

Plate 2

Fig. 5. Field of the myenteric plexus of the upper jejunum incubated for cholinesterase reaction. Three nerve cells show a negative reaction (A) and three other cells show a strong positive reaction throughout their cytoplasm but negative reaction of the nuclei (arrows). The surrounding tissues also give a positive reaction partly obscuring the outline of the cholinesterase positive cells. Adult cat. Frozen section, $20\,\mu$. Acetyl substrate, 2 hr. Light haematoxylin counterstain. $\times 500$.

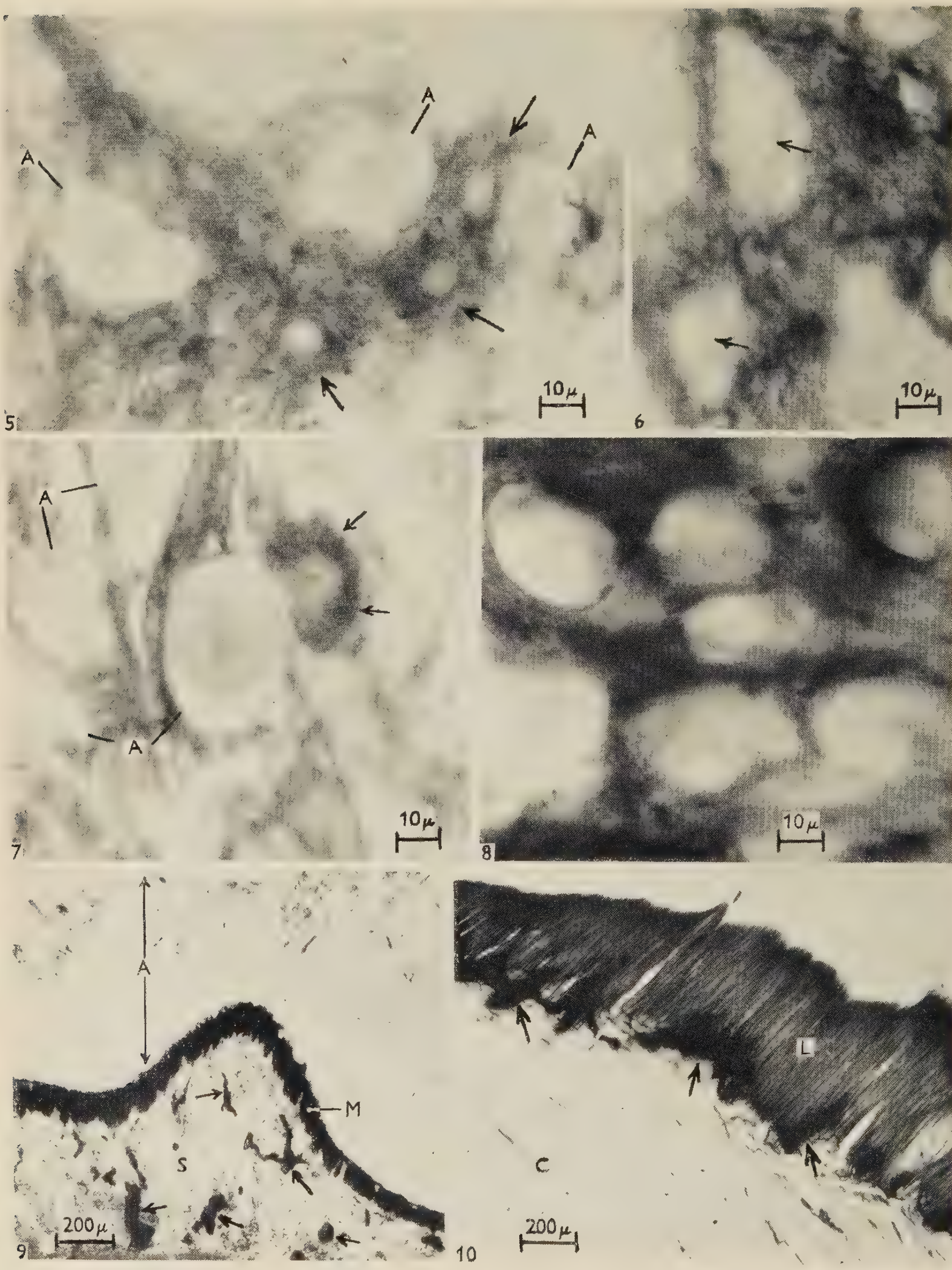
Fig. 6. Field of the myenteric plexus of the upper jejunum incubated for cholinesterase reaction. Nerve cells, recognised by their nuclei (arrows) show a negative reaction, but the surrounding tissues give a strong positive reaction. Adult cat. Frozen section $20\,\mu$. Butyryl substrate, 2 hr. Light haematoxylin counterstain. $\times 500$.

Fig. 7 Field of the superior cervical ganglion incubated for cholinesterase reaction. Several nerve cells show a negative reaction (A), but one cell shows a strong positive reaction in the cytoplasm (arrows). The surrounding tissues show some positive reaction. Adult cat. Frozen section, $20\,\mu$. Acetyl substrate, 2 hr. Light haematoxylin counterstain. $\times 500$.



LEAMING AND CAUNA—MYENTERIC PLEXUS OF THE SMALL INTESTINE OF THE CAT

(*Facing p.* 168)



LEAMING AND CAUNA—MYENTERIC PLEXUS OF THE SMALL INTESTINE OF THE CAT

Fig. 8. Field of the superior cervical ganglion incubated for cholinesterase reaction. All nerve cells show a negative reaction, but the surrounding tissues give a strong positive reaction. Adult cat. Frozen section, 20 μ. Butyryl substrate, 2 hr. Light haematoxylin counterstain. $\times 500$.

Fig. 9. Field of an oblique section of the upper jejunum showing the mucosa (A), muscularis mucosae (M), and submucosa (S) after a prolonged period of incubation for cholinesterase reaction. Some cells of the intestinal glands give a moderately positive reaction, the muscularis mucosae and the nerve plexus (arrows) in the submucosa are strongly positive. Adult cat. Frozen section, 20 μ. Butyryl substrate, 17 hr. No counterstain. $\times 33$.

Fig. 10. Field of an oblique section of the upper jejunum showing the longitudinal muscle coat (L), the myenteric plexus (arrows), and the circular muscle coat (C). Muscle cells of the longitudinal coat and the myenteric plexus are strongly positive, but the circular muscle coat gives an almost negative reaction except for the capillaries which are positive. Adult cat. Frozen sections, 20 μ. Acetyl substrate, 17 hr. No counterstain. $\times 33$.

THE NATURE AND MORPHOLOGY OF THE COSTOCLAVICULAR LIGAMENT

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Some dubiety and contradiction still attend formal accounts of the anatomy of the costoclavicular (rhomboid) ligament, and great variation of appearance is presented by that region of the clavicle which receives the superior attachment of this ligament. These differences in authoritative opinion and in clavicular configuration prompted an inquiry into the structure and nature of the costoclavicular ligament, the results of which are briefly presented here.

MATERIAL AND METHODS

Examination was made of the secondary markings present upon 153 adult (unsexed) clavicles at the site of attachment of the costoclavicular ligament; the findings are listed in Tables 1 and 2 and discussed below. Observation was made upon the ligament and its associated parts during the routine dissection of twenty-five adult cadavers in the Anatomy Department of this College; special dissection was made of the corresponding structures in eight undisturbed embalmed adults and in two unfixed autopsy specimens of the shoulder girdle removed *en bloc*. The costoclavicular ligament was dissected out and its relevant anatomy studied in eighteen species of primate mammal.

From a fresh cadaver a block of tissue was removed which included the clavicle, the first rib, half the sternal manubrium and the upper portion of the scapula with its coracoid and acromial processes. From this block all muscle tissue (save that of m. subclavius) was carefully removed and the various ligaments were subsequently cleaned. The manubrio-costal end of the preparation being fixed, the clavicle was manipulated in various directions and the effect of its excursions upon the costoclavicular ligament was noted.

PREVIOUS ACCOUNTS OF THE LIGAMENT

In British anatomical teaching the costoclavicular or rhomboid ligament was earliest described (Gray, 1858; Humphry, 1858; Thane, 1882) as consisting of a single plane or sheet of fibres, proceeding superolaterally from the first costal arch to the under surface of the clavicle. Later Macalister (1889) described the fibres as ascending medially from the first rib, as did Frazer (1920), who so figured them. The earliest suggestion of the ligament's possibly bilaminar nature came from Morris (1879), who mentioned decussating fibres; his *Human Anatomy* (1907) stated that 'frequently some of the fibres pass upwards and inwards behind the rest and give the appearance of decussating'—a statement retained by Wood Jones (1915).

The first description of a frankly bilaminar costoclavicular ligament was given by

Poirier (1890) and repeated by Poirier & Charpy (1899). For Poirier this ligament was a 'cône tronqué', with anterior and posterior fibre-layers inclined upwards and outwards. Fick (1904) described the anterior fibres as running upwards and outwards and the posterior as running upwards and inwards. Testut (1905) gave no personal account of the ligament, but quoted Sappey as recognizing two component layers therein. Bryce (1915) described two ligamentous laminae, all the fibres of which passed upwards, outwards and backwards. More recently, Wood Jones (1949) and the centenary edition of *Gray's Anatomy* (1958) gave a simplified account of the two ligamentous planes described by Fick.

Those authorities who admit the presence of two component layers in the costoclavicular ligament are not unanimous concerning the presence of an interposing bursa, where they do not entirely ignore such a structure. Poirier (1890) alone described this bursa fully: he regarded it as constant, though of variable nature, with a lining which might be 'smooth and glistening or rough and reddish'. His account was adopted by Fick (1904). Testut (1905) quoted Sappey's description of a bilaminar ligament having 'lax, cellular tissue between the layers and sometimes a bursa'. Bryce (1915) regarded this bursa as inconstant, but adduced no personal evidence; Wood Jones (1949) was likewise content virtually to adopt Poirier's findings.

OSTEOLOGICAL EVIDENCE

The 153 clavicles examined revealed distinctive differences in the pattern of the canonical 'rhomboid impression'. Most commonly the costoclavicular ligamentous area was flat; in a considerable number of specimens a distinct pit or depression was present; in a smaller proportion of specimens the relevant area showed an

Table 1. *Nature of costoclavicular area on clavicle*

No. of clavicles examined	Flat	Depressed	Elevated	Rough	Smooth	Ant. groove	Post. groove	Ant. lip	Post. lip
Right, 78	41	28	9	40	38	3	2	7	25
Left, 75	52	15	8	48	27	3	2	10	35
Total, 153	93	43	17	88	65	6	4	17	60

Table 2. *Characteristics of costoclavicular area*

Combined characters	Right	Left	Total	Percentage of 153 clavicles
Flat and rough	20	28	48	31
Flat and smooth	21	24	45	29
Depressed and rough	15	12	27	18
Depressed and smooth	13	3	16	10·5
Elevated and rough	5	8	13	8·5
Elevated and smooth	4	0	4	2·6

elevation (see Table 1). In most clavicles this ligamentous area was rough, in a minority it was smooth (Table 1). The proportions of combinations of the main features noted are given in Table 2, whence it appears that 60 % of clavicles manifest a 'flat' rhomboid area, 30 % a depression here, and 10 % an elevation of the

bone at this site. The ligamentous area is invariably oval in outline and additionally it may be provided, anteriorly or posteriorly, with either prominent 'lips' or limiting grooves (see Table 1 and Fig. 1).

These findings differ from those of Poirier, who described the costoclavicular attachment area as having most commonly the form of a rough oval eminence, though sometimes the form of an oval fossette, and as being sometimes poorly marked. His suggestions that these osteological differences are due to variations in

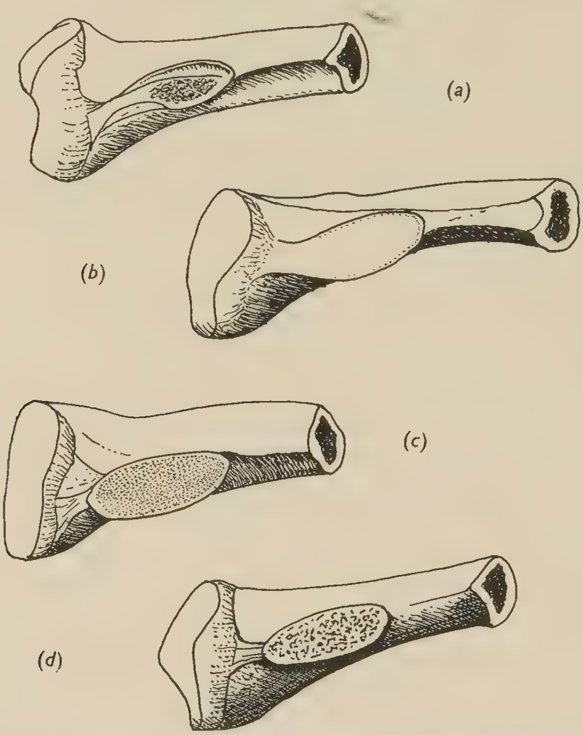

Fig. 1. *Homo.* Varieties of costoclavicular area on clavicle. a = rough, flat area with posterior lip; b = smooth and flat area; c = smooth, depressed area; d = rough, elevated area.

the degree of development of the ligament or to methods of specimen preparation appear to be inadequate; no other attempt at explanation of these differences has, however, been encountered in the literature.

The markings made by the costoclavicular ligament upon the clavicle indicate clearly that medially the ligament must merge with the sternoclavicular joint capsule and that laterally the anterior and posterior components of the ligament are in continuity.

In one clavicle examined the costoclavicular attachment area had the form of a smooth, elevated, faceted apophysis, which established diarthrodial articulation with a corresponding faceted apophysis on the first rib. Poirier (1890) stated that such a variation occurred once in ten specimens (the costoclavicular ligament forming the capsule of the diarthrosis), but he furnished no supportive statistical evidence. In the present study, only four of 153 clavicles (Table 2) manifested the smooth,

elevated apophysis associated with a costoclavicular diarthrodial arrangement. Fick (1904) referred to examples of diarthrodial union between first rib and clavicle reported by Poirier, Luschka, Cruveilhier and Waldeyer, without, however, any mention of statistical incidence. Wood Jones (1949) gave a 10% incidence of the occurrence of costoclavicular diarthroses, but his statement appears to be merely a numerical modification of Poirier's.

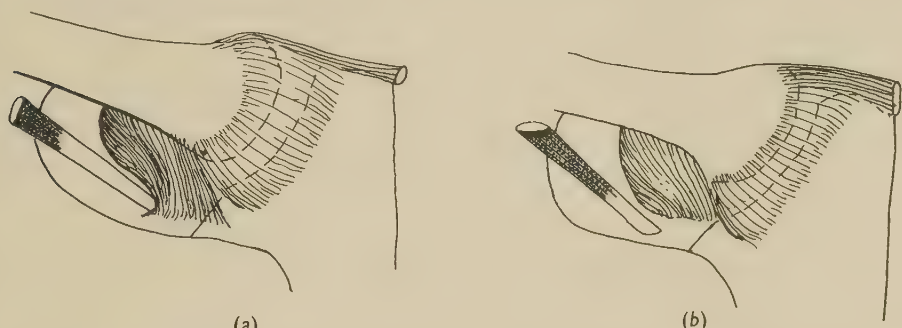

Fig. 2. *Homo*. Costoclavicular ligament in relation to sternoclavicular capsule and subclavius tendon. a = the more usual arrangement; b = subclavius tendon anterior to the ligament.

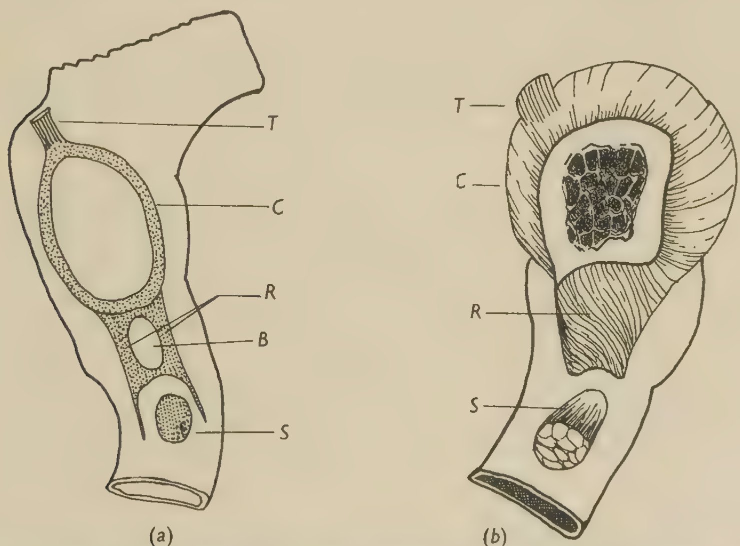

Fig. 3. *Homo*. Mutual relationships of sternoclavicular capsule, costoclavicular ligament and subclavius tendon, a in diagrammatic superior view, b from the lateral aspect. B = costoclavicular bursa; C = sternoclavicular capsule; T = interclavicular ligament; R = costoclavicular ligament; S = subclavius.

ANATOMY OF COSTOCLAVICULAR LIGAMENT

The costoclavicular ligament is disposed as an inverted, truncated cone, flattened antero-posteriorly (Figs. 2, 3). It averages half an inch in length (or height), three-quarters of an inch in maximal (superior) width and half an inch in thickness. It is not a uniformly solid structure, but is cavitated by the 'bursa' described below. The

walls of this bursal cavity are the anterior and posterior laminae of ligamentous fibres, which are mutually continuous laterally so forming the lateral bursal wall; medially also the ligamentous laminae fuse and abut against the lateral aspect of the sternoclavicular capsular ligament, with which they are continuous. Inferiorly, the apex of this conical structure is attached to the first rib adjacent to the first costal cartilage and usually extends on to the cartilage itself. Superiorly, the ligament is attached to the margins of the oval receptive area on the clavicular 'neck', the fibres gaining insertion into such circumscribing lips or grooves as may there be present.

In the anterior lamina of the ligament most fibres pass upwards and outwards, the innermost ones being the more vertically disposed; the posterior laminar fibres pass upwards and inwards. The most lateral of the anterior fibres run upwards and backwards to blend with those of the posterior lamina, conferring upon the ligament a twisted appearance in norma lateralis. (Scant attention has been accorded this feature of the ligament: Morris (1907) noted indeed the 'appearance of decussation' and though Fick (1904) gave a detailed account of fibre directions he failed to observe the lateral continuity of the anterior and posterior laminae.)

Medially the ligament is closely applied to the lateral aspect of the sternoclavicular joint capsule, usually without any discernible free border or discontinuity (Fig. 3). In one only of thirty-five subjects dissected was the ligament bilaterally separated from the capsule by some loose fibro-fatty tissue. Fick (1904) stated that frequently the outermost portion of the synovial sac between the intra-articular disc and the clavicle is pinched off to form an independent mucous bursa, which then lies antero-medial to the costoclavicular ligament. No such bursa was observed however during the present investigation.

The 'bursa' of the costoclavicular ligament is invariably present, but its parietes and contents manifest some variation. Occasionally the cavity has a smooth, shining lining akin to synovial membrane and contains a quantity of thin viscid fluid. (This type of bursa would appear to be associated with a smooth, or even smooth and elevated, costoclavicular area on the clavicle.) More frequently, however, the bursal lining is shaggy and irregular, and the cavity is filled with minute lobules of fatty material interspersed with loose fibres and a little free fluid. On no occasion has the bursal cavity been found to communicate with that of the sternoclavicular joint, contrary to Poirier's (1899) finding and Wood Jones's (1949) statement.

RELATIONS OF COSTOCLAVICULAR LIGAMENT

The tendon of origin of m. subclavius lies immediately lateral to the inferior (or apical) attachment of the costoclavicular ligament (Fig. 3, *a*, *b*) and the tendon may indent the ligament's lateral aspect. Very commonly the sheath of m. subclavius gains attachment to the front and back of the ligament which thus forms the medial extremity of that sheath. In some 20 % of subjects the subclavius tendon is attached anterior to the costoclavicular ligament (Fig. 2*b*), and the two layers of the muscle sheath become lost in the loose connective tissue anterior to the ligament. Occasionally also the subclavius sheath is much thickened medially so that its differentiation from the ligament may be impracticable.

Fick (1904) quoted Henle as including in the anterior lamina of the costoclavicular ligament fibres which run anteriorly to the subclavius tendon in direct continuity with the subclavius fascia and as therefore regarding the subclavius tendon to be enveloped by the ligament. Since the conditions referred to by Henle do not invariably obtain, and as judgement herein must often be arbitrary, it would be better to regard the fibres in question as representing merely a thickening of the subclavius muscle sheath.

Whereas continuity of costoclavicular ligament and sternoclavicular capsule is the rule, continuity of the ligament with the subclavius tendon is never encountered, and the same holds good for the arrangement of the corresponding structures in non-human Primates. There is thus no anatomical justification for the view advanced by Bland Sutton (1897) that the costoclavicular ligament represents a degenerated portion of the m. subclavius.

FUNCTIONS OF COSTOCLAVICULAR LIGAMENT

Elevation of the pectoral girdle is limited by this ligament. During clavicular elevation the costoclavicular ligament becomes tense and then acts as a fulcrum, while a further limited gliding of the clavicular 'head' takes place in an infero-lateral direction, a movement finally arrested by the postero-superior fibres of the sternoclavicular capsule.

Depression of the clavicular lateral extremity produces compression of the costoclavicular ligament between clavicle and first rib. (When apposed clavicular and costal apophyses exist, their actual contact is ensured by this movement and the bursa functions as the synovial component of a diarthrodial joint.) Again the costoclavicular ligament acts as a fulcrum and further depression of the shoulder region is limited by the interclavicular ligament and the intra-articular meniscus.

Protraction of the clavicular lateral extremity produces a limiting tension in the posterior laminar fibres of the costoclavicular ligament and in the anterior capsular fibres; retraction conversely produces a limiting tension of the anterior laminar fibres of the ligament and in the posterior capsular fibres; the axis of movement in each case is vertically through the clavicle between ligament and capsule. Clavicular rotation in the long axis is limited by the costoclavicular ligament—backward rotation of the clavicular 'head' by its anterior, and forward rotation by its posterior, fibres.

The costoclavicular ligament is the effective inferior ligament of the sternoclavicular joint (as appreciated by Henle, Poirier, Testut and Fick), and is capable of maintaining clavicular stability even after division of the joint capsule and its contained meniscus. As a whole it resists upward displacement of the clavicle 'head'; it likewise counters the upward pull of the clavicular head of m. sternomastoideus and the lateral pull of the clavicular portion of m. pectoralis major.

Johnston's (1909) statement that the costoclavicular ligament is 'always tense, even when the upper extremity is hanging by the side' is not confirmed by present observations, which discover the ligament, under such conditions, to be lax and frequently, indeed, to form a cushion between clavicle and first rib. Only at the end of a particular range of movement does the ligament become maximally tense. Johnston also stated that the costoclavicular ligament was responsible for clavicular

elevation being permitted by an upward rotation of the anterior surface of the bone; but observation on the above-mentioned special preparation showed that pure elevation can and does occur independently of any such rotation.

COMPARATIVE ANATOMY

The literature of comparative anatomy contains remarkably little concerning the costoclavicular ligament, syndesmology being generally the most neglected system in comparative studies of either particular forms or natural groups. This paucity of information applies notably to the Primates (*sensu lato*). The canonical zoological treatises afford no information, and special monographs disappointingly little.

Thus Dobson (1882–90) on the Insectivora in general, Le Gros Clark (1926) on *Ptilocercus lowii*, Woollard (1925) on *Tarsius spectrum*, Beattie (1927) on *Hapale jacchus*, Sonntag (1923, 1924) on *Pan satyrus* and *Pongo pygmaeus*, and Raven (1950) on *Gorilla gorilla* omit all reference to the costoclavicular ligament. Ayer (1948) notes its presence in *Semnopithecus entellus* and its continuity with the sterno-clavicular joint capsule. Osman Hill (1953–57) states only that 'in some Platyrrhini (*Ateles*, according to Parsons) the chief synovial articulation is between clavicle and first rib, but usually the clavicle is connected to the first rib only by the accessory (rhomboid) ligament, which is well developed in *Tarsius* and monkeys, being connected also to the capsular ligament in the former'. He is silent regarding the costoclavicular ligament in Pithecoidea.

In view of such scantiness of available comparative information, the anatomy of the costoclavicular ligament was investigated in the non-Primate hedgehog (*Erinaceus europaeus*) and Egyptian fruit bat (*Rousettus aegypticus*), and in the following Primates: ringtailed lemur (*Lemur catta*), Bosman's potto (*Perodicticus potto*), thicktailed bushbaby (*Galago crassicaudatus*), slow loris (*Nycticebus coucang*), tarsier (*Tarsius spectrum*), marmoset (*Hapale jacchus*), squirrel monkey (*Saimiri sciurea*), weeper capuchin (*Cebus apella*), woolly monkey (*Lagothrix humboldtii*), red-handed tamarin (*Mystax midas*), howler (*Alouatta seniculus*), crabeating macaque (*Macaca irus*), mona monkey (*Cercopithecus mona*), patas monkey (*Erythrocebus patas*), black and white colobus (*Colobus polykomos*), silvery gibbon (*Hylobates lar leuciscus*), orang (*Pongo pygmaeus*) and chimpanzee (*Pan satyrus*). Two specimens each of tarsier, potto, marmoset, tarmarin and howler were dissected; the orang and chimpanzee were young animals; in all specimens the ligaments of the two sides were dissected.

In all these forms the costoclavicular ligament manifested a striking uniformity of conformation and was clearly nothing more than the functionally specialized inferior component of the sternoclavicular joint capsule. In the specimens of *Erinaceus*, *Galago*, *Loris*, *Tarsius* and *Saimiri* examined, the ligament (Fig. 4*a*, *b*) was in nowise specially distinguishable from that capsule: in *Rousettus*, where the capsule was notably thin, the costoclavicular ligament (Fig. 5*a*) was prominent and extremely well developed; in the other forms studied its anatomical entity was sufficiently apparent. In the gibbon, orang and chimpanzee specimens the ligament was particularly wide; in the *Perodicticus*, *Hapale* and *Alouatta* specimens (Fig. 6) it was bifascicular; in the *Macaca irus* specimen alone was it somewhat separated from the

sternoclavicular capsule by a forward herniation of the synovial lining thereof as a small intervening bursa; in *Saimiri* and in *Mystax* the cavity of the sternoclavicular joint and the bursa of the costoclavicular ligament were continuous and the costoclavicular ligament was thereby rendered partially bilaminar. Otherwise the attachments and relations of the costoclavicular ligament, in the forms examined, displayed an almost monotonous anatomical similarity.

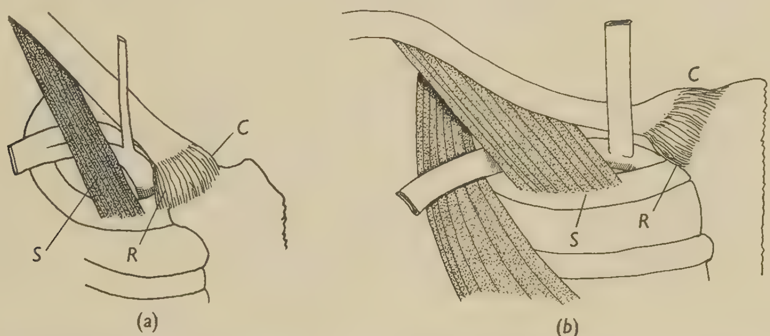

Fig. 4. Unemphatic type of costoclavicular ligament (the ligament being an undifferentiated portion of the sternoclavicular capsule) in (*a*) *Erinaceus europaeus*, (*b*) *Tarsius spectrum*. (Labelling as in preceding figure.)

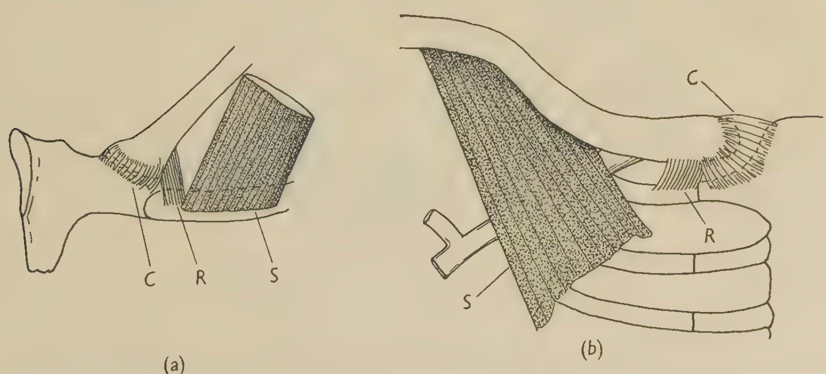

Fig. 5. Emphatic type of costoclavicular ligament, in (*a*) *Rousettus*, (*b*) *Hylobates*. (Labelling as in preceding figures.)

The complete morphological independence of the costoclavicular ligament from the m. subclavius was emphatically apparent in every specimen of every form examined. There was never evident the slightest sign (or even hint) of continuity between this ligament and the subclavius tendon. The ligament is demonstrably a derivative of the capsule of the sternoclavicular articulation and no valid anatomical basis exists for the Bland Sutton (1897) hypothesis which would derive the costoclavicular ligament by degeneration from the subclavius muscle.

The human costoclavicular ligament is distinguished by its relatively large size, its conical or cylindrical fibre-disposition, its bursal cavity and its attempted modification (occasionally successful) towards a diarthrodial joint.

Factors responsible for the development of an emphatic costoclavicular ligament

would appear to be: (*a*) wide range of clavicular movement, (*b*) the necessity for strengthening inferiorly the sternoclavicular articulation, (*c*) the habitual posture of the trunk.

In the Chiroptera and the higher Primates at least the forelimb is relieved of much of the mechanical burden of supporting the body weight and is endowed with an unusual range of independent movement for flight (Chiroptera) or prehension. Under such new physical conditions as the sternoclavicular joint must consequently

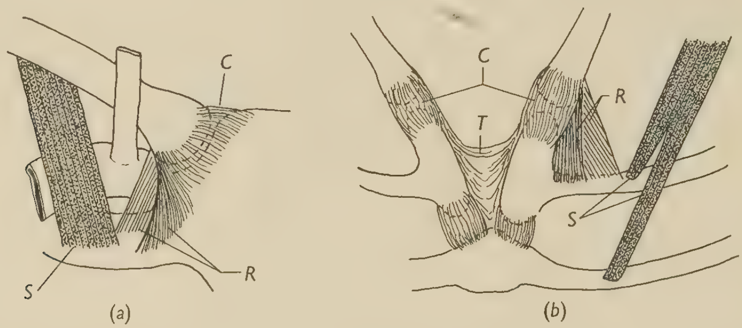

Fig. 6. Bifascicular type of costoclavicular ligament, in (*a*) *Hapale*, (*b*) *Alouatta*. (Labelling as in preceding figures.)

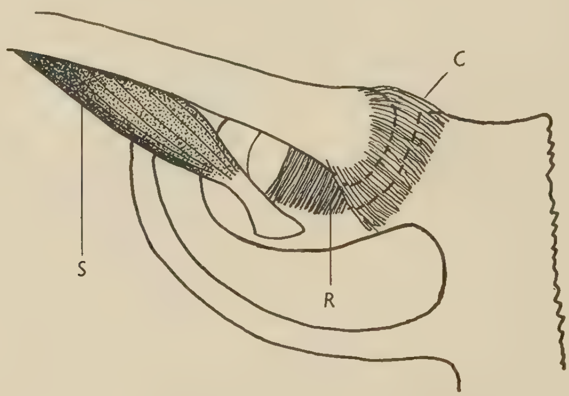

Fig. 7. Wide emphatic form of costoclavicular ligament in *Pongo*. (Labelling as in preceding figures.)

meet, the costoclavicular ligament acquires an enhanced development and importance as the necessary stabilizer of the fulcrum situate alongside the clavicular head, and becomes more readily recognizable as an anatomical entity.

Nevertheless, despite forelimb 'emancipation', in no subhuman Primate is the forelimb not employed, to some degree and on some occasions, as a supportive, rather than a prehensible, organ. (Probably the Gibbon alone dispenses habitually with forelimb body support.) And in no Primate save man is the trunk borne habitually erect and the forelimb completely relieved of all engagement in habitual stance or progress. Hence, in man, an ensuing qualitative difference in the mechanics of the sternoclavicular joint, the necessity for a particular inferior strengthening of

that joint and the appearance of the distinctively human costoclavicular ligament—an ancestral syndesmosis attempting functional modification in the direction of a diarthrodial joint.

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DIETARY FACTORS AND NERVE FIBRE MYELINATION

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INTRODUCTION

Despite numerous investigations by morphological as well as by physiological methods, the peripheral nerve still harbours many problems. A considerable amount of attention has been centred particularly on the fibre calibre, yet we lack a valid explanation as to the purpose of fibres of different diameters.

The total diameter of a myelinated fibre, as seen on a cross-section, incorporates two chief constituents; the axon and its sheath of compact myelin. While at one time a fixed relationship has been postulated between these two parts evidence to the contrary has been accumulating. In their recent paper on axon-myelin relationship in peripheral nerve fibres, Sunderland & Roche (1958) have defined clearly the various aspects of this problem and have covered the related literature. As a result of their own investigation they emphasize that nerve fibres of identical diameters may yet have myelin sheaths of different thickness, and furthermore, that myelin sheath thickness followed over some length on the same fibre may vary. It is the purpose of this paper to point out that axon-myelin relationship is also subject to changes of a dynamic nature.

MATERIAL AND METHODS

The material for this investigation consisted of the sciatic nerves of rats subjected to various diets at the age of $2\frac{1}{2}$ months for a period of 8 weeks. The animal's weight at the beginning of the experiment was 220 ± 20 g.; all were male and of the same age. Findings from the same material, considering the total diameters of the nerve fibres only, have been published previously (Tomasch, 1959).

This report deals with the effects of diets rich or poor in the basic foodstuffs, protein and fat. Contrasted against each other are the results of measurements on the sciatic nerves of five groups of five animals each; one group having received protein-free diet consisted of only four animals, as one rat had died early during the dieting period. The diets, free of protein and free of fats, were purchased from Nutritional Biochemicals Co., diets high in protein and high in fat consisted of ordinary rat biscuits liberally supplemented with meat or suet and lard, respectively. There was also one group of five rats acting as a control.

Sections of the sciatic nerves taken at the level just below the greater sciatic foramen were examined. These sections were stained by Weigert's and Häggqvist's methods, respectively. Measurements were carried out on photomicrographs of these sections using a final magnification of 1350. Using fibres of round circumference only, the total and axon diameters were measured. Results of measurements

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from each group of rats were pooled together and treated as an entity, to compensate for possible individual variation.

At the level examined the sciatic nerve of the rat is divided into two bundles, a large medial popliteal and a small lateral popliteal portion; and, as in man, these two bundles do not seem to exchange any fibres. The bundle to become the medial popliteal nerve comprises about $\frac{2}{3}$ of the total cross-sectional area of the sciatic nerve. It is a single bundle and not further fasciculated. The myelinated fibres of various diameters stained by Weigert's method may not be distributed uniformly throughout this bundle, however, there is also no obvious grouping of any fibre size in any particular area of the cross-sections. Measurements of random samples of fibres were attempted by the taking of fifteen high-power photomicrographs in a clockwise direction, twelve pictures taken at positions according to the figures of the clock dial and three from the central portion.

The staining methods employed were Kultschitzky's modification of Weigert's original method and Häggqvist's modification of Alzheimer-Mann staining method. Both methods have in common the pre-treatment of the nerves before embedding. Fixation was carried out in 10 % neutral formalin for 1 week. Then the nerves were rinsed in running tap water for 1 hr. and, after washing, mordanted in a 5 % aqueous solution of potassium bichromate for 2 weeks. Dehydration was effected by rising concentrations of alcohol. Xylol was used as a clearing agent before embedding in paraffin. All steps from fixation to staining were carried out simultaneously, and all solutions used were prepared in advance for the whole material.

Adjacent sections, 7 μ thick, were stained by the two above-mentioned methods. For the Weigert method sections were stained for 36 hr. in the alcoholic haematoxylin solution and differentiated for 4 hr. in a mixture of ten parts of concentrated aqueous lithium carbonate solution and 1 part of a 1 % aqueous potassium ferricyanide solution. This fluid was changed three times at hourly intervals. For Häggqvist's method, sections were treated for 24 hr. in a saturated aqueous solution of phosphotungstic acid and, following a brief rinsing in distilled water, stained for 24 hr. in the staining mixture of methyl blue and eosin. This stain was differentiated by three changes of 95 % alcohol over a 1 min. period.

Photomicrographs of the stained sections were taken on 35 mm. Plus X film using a 100 × oil immersion lens in combination with a 6 × eyepiece. The negatives were enlarged on medium contrast paper to a final magnification of 1350.

A special method was devised for the taking of measurements amounting to fractions of microns only. At the magnification employed, 1 μ was enlarged to 1·35 mm. on the photomicrographs. A ruler was made dividing this distance of 1·35 mm. into ten parts, making it thereby possible to measure one-tenth of a micron. The finely divided scale for the reading of the mantissa of logarithms on a slide rule was photographed for this purpose. The distance between 100 such fine divisions was reduced photographically to a length of 13·5 mm. This finely divided scale was mounted on the stage of a wide-field low-power dissecting microscope. Finely pointed calipers were used for taking measurements from the photomicrographs of the sections. By applying these calipers to the mounted scale under the dissecting microscope, readings to the nearest tenth of a micron were possible. It would have been feasible to judge visually fractions of this distance in most cases

but only tenth of microns measurements were recorded. By this co-ordination of ruler and magnification of photomicrographs no calculations were needed, results could be read directly in microns and their fractions.

The axon diameter and total diameter of about 100 fibres from all over the cross-section of the medial popliteal nerve of each rat were measured on the Weigert stained sections, this amounting to 500 fibres for each kind of diet.

The sections stained with Häggqvist's method were used to determine the distribution of fibre sizes generally, inclusive of unmyelinated fibres. Total diameter measurements were carried out on the photomicrographs of these sections to whole microns only. However, 500 such measurements were taken on each medial popliteal nerve cross-section amounting to about 2500 fibres for each diet. On the photomicrographs of these sections stained by Häggqvist's method, *all* fibres found within sample areas of 40 by 70 μ were measured. For this purpose not only the photomicrographs were used, but the areas in question were scrutinized at the same time under the microscope, particularly for the identification of fibres of small diameter and of unmyelinated fibres. The resulting fibre size distribution curves were needed to gain information about the relative frequency of the various fibre sizes.

RESULTS

Consideration may first be given to the three histograms of Fig. 1, depicting the fibre size distribution in the sciatic nerves of the protein-free, the high protein dieting groups of rats and a control group, respectively. Each histogram is the result of more than 2500 fibre measurements of total diameter only; the sections having been stained by Häggqvist's method. In all three histograms the first mode is predominantly due to unmyelinated fibres, as indicated by the shaded area; only a few myelinated fibres as small as 1–2 μ in diameter may be expected; the latter are indicated by the unshaded portion. The second mode, caused by myelinated fibres only occurs at 5–6 μ in all three histograms. However, there are differences as to the largest fibres present. Those from the protein-free dieting animals reach sizes up to 11 μ only, 2 μ less than in the high-protein dieting animals and 1 μ less than in the control group. In the protein-free dieting group the second mode is higher, incorporating obviously the fibres which reached larger diameters in both the other groups.

Use of the χ^2 distribution for comparison of the three histograms has resulted in χ^2 values in excess of the table for $P = 0{\cdot}001$. According to this method of testing and regarding the outside diameter of the nerve fibres only, there is a significant difference between the fibre-calibre distributions of the controls on the one side and the high-protein dieting or protein free dieting animals respectively on the other side.

Following these initial observations, measurements have been taken, from each of the three groups of rats, of about 500 fibres, on sections stained by Weigert's method. In the scatter diagram, Fig. 2, are plotted total diameters of fibres against their axon diameters, in the protein-free and protein-rich dieting groups only. The control group is not shown as its measurements, though lying between the two extreme groups, obscure the principles to be demonstrated.

While plotting this diagram it has become increasingly obvious that at a given total diameter, a tendency exists for the axon diameters in protein-free dieting animals to be higher than in the nerve fibres of the protein-rich dieting rats. This tendency is quite clearly expressed in the scatter diagram by the higher position of the circles used for denoting the values in the protein-free group. While the extreme values in either group are clearly distinct, the intermediate ones overlap considerably. It remains therefore for statistical methods to evaluate the significance, if any, of this tendency.

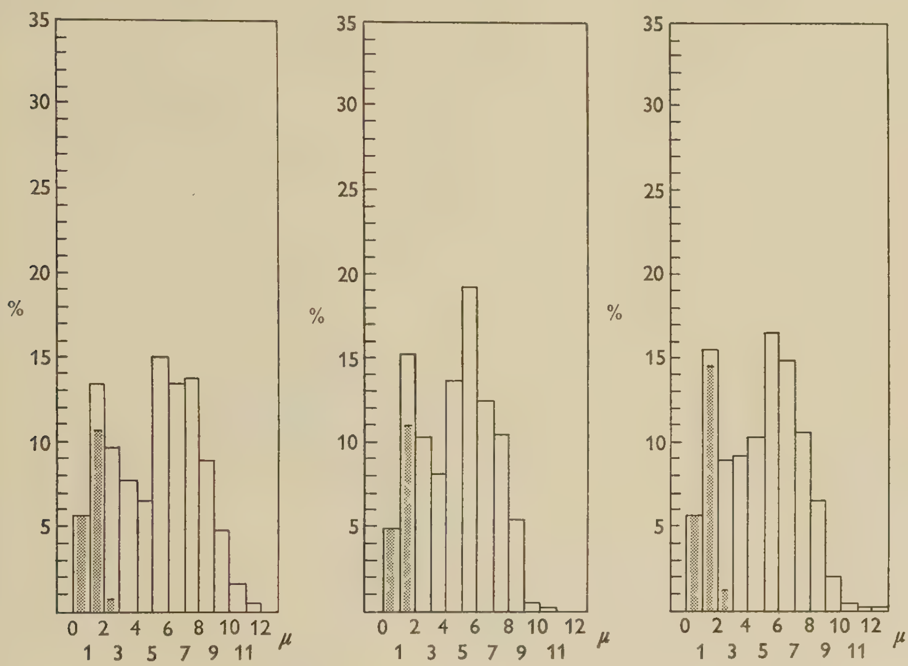

Fig. 1. Fibre-size distribution histograms. Control group (left), protein-free dieting group (middle and protein-rich dieting group (right). Shaded areas indicate proportion of unmyelinated fibres.

The outward effect on the four rats of protein depletion of 2 months' duration has been mainly loss of weight; it has dropped from an average of 220–128 g. Yet the animals have remained lively for a considerable period, growing apathetic during the last 2 weeks only. The five rats receiving a diet liberally supplemented with meat have gained weight to an average of 395 g. One is therefore comparing the effects of diets which in one group have caused a loss of weight of 42 % and in the other a gain of 79 %. Still the structural differences as expressed in terms of myelin sheath thickness are so minute as to become measurable only by employing a high microscopic and photographic magnification, thereby expressing the tardiness of nervous tissues in responding quantitatively to nutritional influences.

The first step at statistical evaluation of the data collected consists of the calculation of the weighted arithmetical means for the axon diameters in each class of outside diameters; each class comprising the range of 1 μ. The mutual positions of

the respective means (Fig. 3) reflect the tendency of the two variates and also permit recognition of the non-linear trend in the plotted axon-total diameter relationship. This non-linear trend is expressed best by an equation of the power function type. A regression of this kind is calculated by a least-squares method. An index of correlation of 0·989 has been taken to express an excellent fit of the calculated regression for the data.

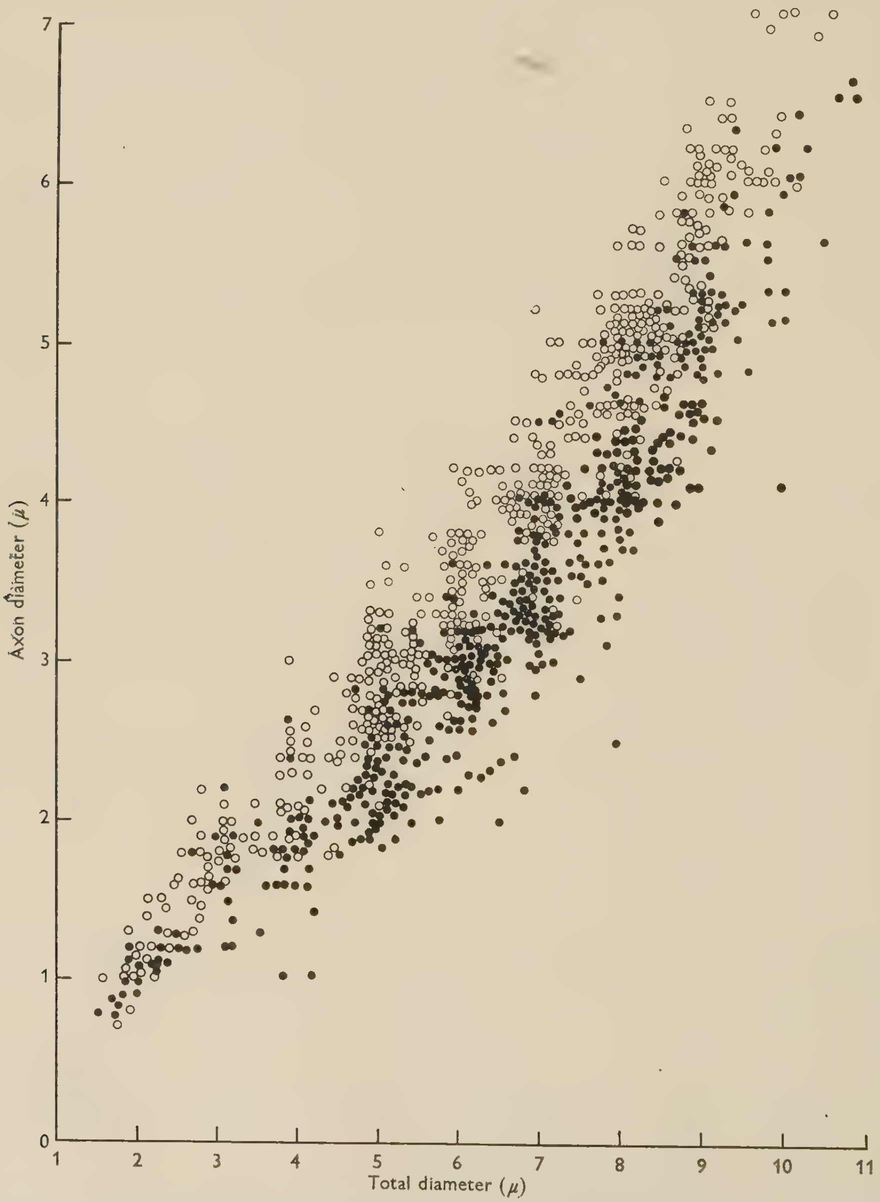

Fig. 2. Scatter diagram for comparison of results of measurements from nerve fibres of protein-free dieting rats (circles) and protein-rich dieting rats (dots).

The distance between the means within each class amounts to about one and a half the respective standard deviations. This permits a rough calculation that about 7 % of the values in each dietary group, that is the upper extreme ones for the protein-free diet and the lower extreme ones for the protein-rich diet, are by way of comparison significant for this particular diet. Further statistical analysis has been based on the null hypothesis that the two variates are actually samples of one and the same population. Although the trend of the variates is decidedly non-linear, the deviation from linearity is minimal as expressed by exponents of 1·03 or 1·06. This minimal deviation from linearity has made it possible to subject the data to an analysis of covariance, fitting linear regressions for this purpose.

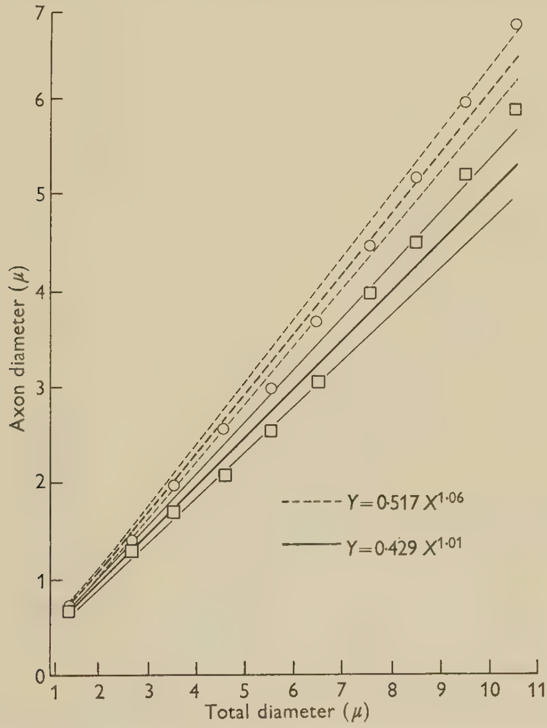

Fig. 3. Calculated regression lines for the measurements from nerve fibres of protein-free dieting rats (heavy dashes), protein-rich dieting rats (heavy solid line). Standard error of the estimate is indicated for protein-free diet (light dashes) and protein-rich diet (light solid lines). Circles denote arithmetic means of protein-free diet, squares of protein-rich diet.

This method of testing has been used to compare the effects of the two dietary extremes. Between the measurements taken from the nerve fibres of the high-protein and protein-free dieting rats, the analysis of covariance has resulted in a value of $F = 6{\cdot}65$. This figure is well in excess of the table value for $F_{0{\cdot}05} = 4{\cdot}49$, falling short, however, of the value for $F_{0{\cdot}01} = 8{\cdot}53$, thus permitting the conclusion that there is a distinctiveness in the two sets of measurements and it is suggested that this difference is due to the diets employed.

The group of rats receiving a diet high in fat gained the same average weight as

the control animals. The other five rats dieting fat free gained moderately in weight from 220 to 268 g. We are therefore comparing two groups, both of which gained weight, one 21 %, the other 60 %. In Fig. 4 are shown the fibre size distribution curves of animals on these diets and the control group. In the case of the fat-free diet group the largest fibres present are 2 μ smaller than in both the high-fat dieting rats and the controls. As was also the case in the protein free diet, the second mode at the 5–6 μ class is higher in the fat-free group and the myelinated fibres contained

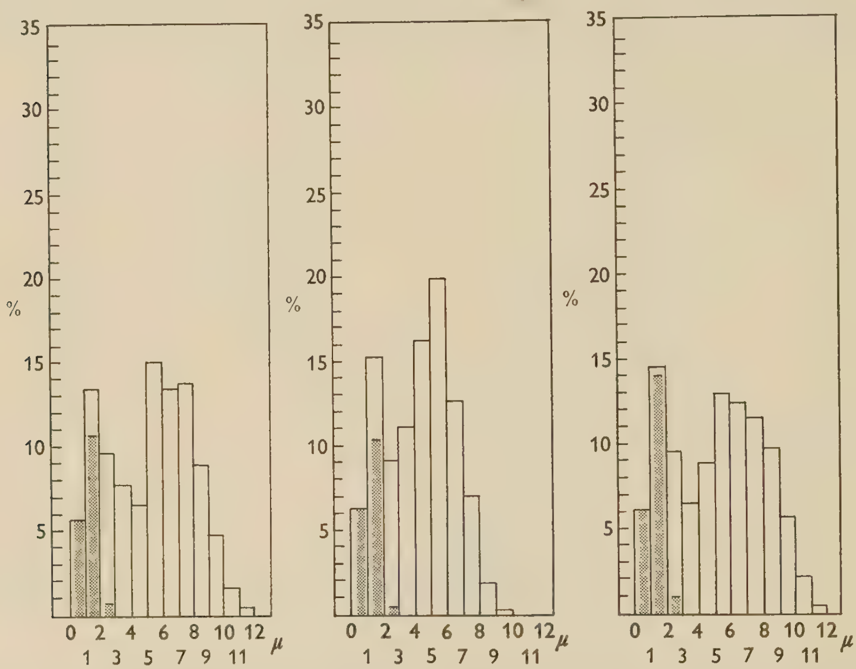

Fig. 4. Fibre size distribution histograms, control group (left), fat-free dieting group of rats (middle) and fat-rich dieting group of rats (right). Shaded areas indicate proportion of unmyelinated fibres.

in the second mode of the distribution curve are seemingly more condensed. As in the case of the protein-free and high-protein diets, these histograms are constructed from total-diameter measurements of more than 2500 fibres taken on sections stained by Häggqvist's method.

The three histograms of Fig. 4 have been compared by means of the χ^2 distribution. The values in both the fat-free as well as fat-rich dietary groups, tested against the values obtained from the control group, show a significant difference $P = 0{\cdot}001$ compared with those in controls.

Again, measurements of total against axon diameters have been carried out and plotted in the scatter diagram (Fig. 5). There is a tendency for the values from the fat-free diet to fall at higher levels of axon diameter. This tendency has been tested again by the reliability measure employed for the protein free and high protein groups.

The weighted arithmetical means fall at slightly smaller intervals than has been the case in the free and high-protein groups. Consequently the calculated regression lines (Fig. 6), are closer with less tendency to diverge. Following the same assumptions as in the case of the protein-free and high-protein diets an analysis of covariance has been performed, testing the two dietary extremes. This analysis between the fat-free and fat-rich dietary groups has resulted in a value of $F = 4{\cdot}97$. This figure is only slightly in excess of the table value $F_{0{\cdot}05} = 4{\cdot}49$.

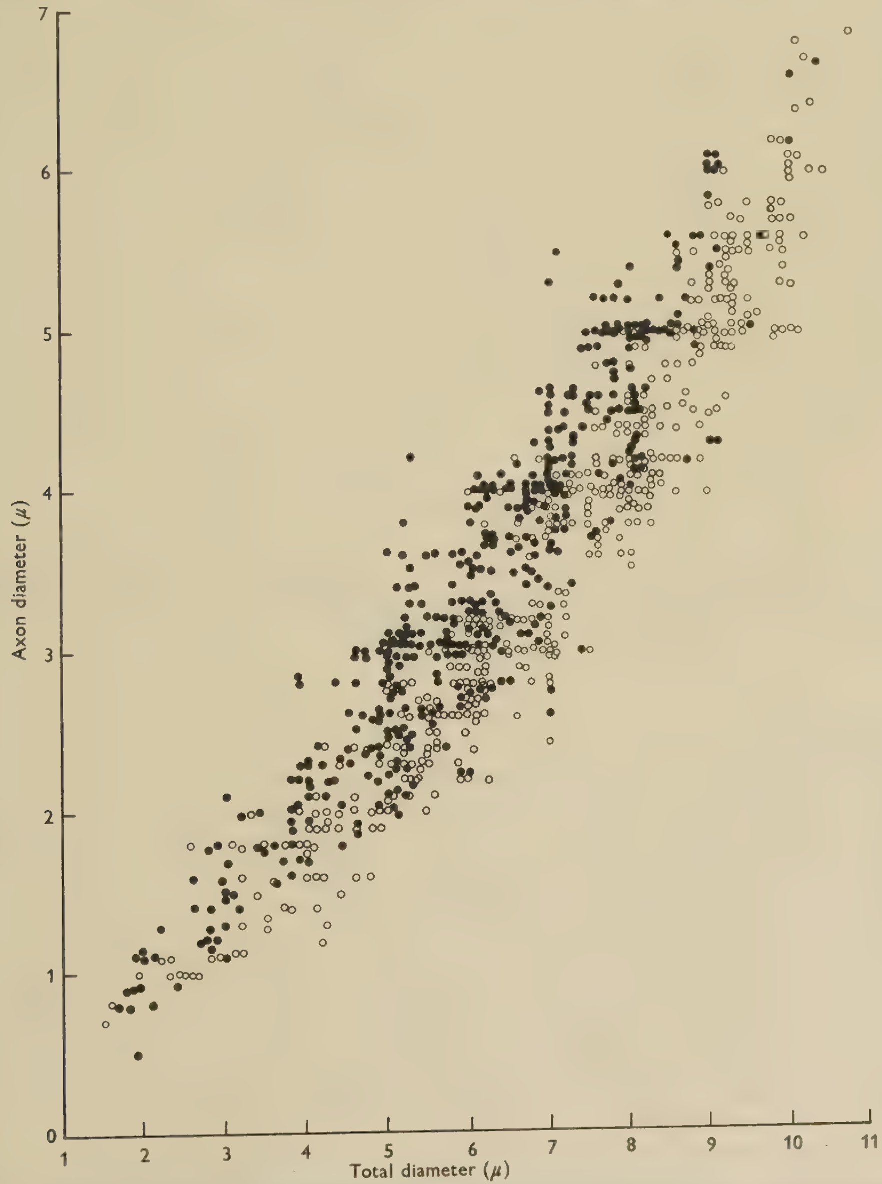

Fig. 5. Scatter diagram for comparison of measurements from nerve fibres of fat-free dieting group of rats (dots) and fat-rich dieting animals (circles).

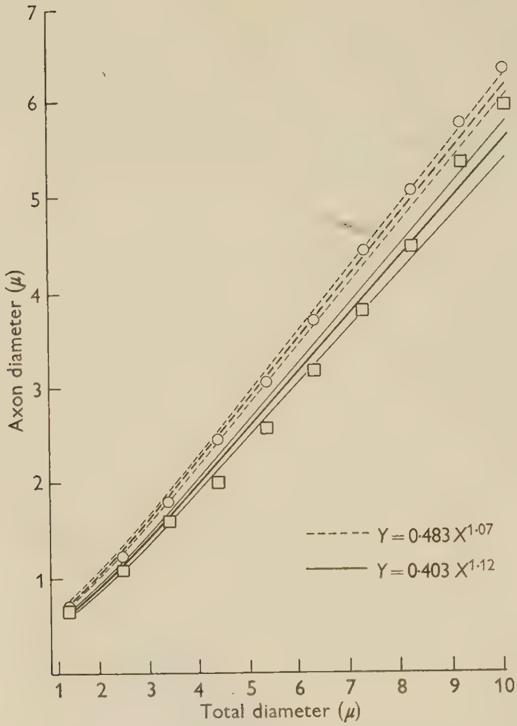

Fig. 6. Calculated regression lines for the measurements from nerve fibres of fat-free dieting rats (heavy dashes) and fat-rich dieting rats (solid line). Standard error of the estimate is indicated for fat-free diet (light dashes) and fat-rich diet (light solid lines). Circles denote positions of arithmetic means of fat-free diet, squares of fat-rich diet.

DISCUSSION

The differences obtained on comparing axon diameters in animals subsisting on dietary extremes in contents of proteins and fats appear to be real as proven by application of statistical reliability measurements. While undoubtedly some quantitative component of myelination has been altered, there is no evidence in these data as to the exact nature of such alteration. However, certain features of the differences observed permit conjecture as to its nature, at least as guide for the planning of further experiments.

While there is a great similarity in the results from the two groups of diets, the differences resulting from diets with and without proteins are more pronounced than from fat-free and fat-rich diets. The most likely reason for this greater effect of the protein-test diets lies in the greater weight loss or weight gain between the two groups; the weight difference being 266 g., as compared with only 85 g. between animals on fat-free and fat-rich diet. It can even be argued that the differences recorded in the nerve fibres could be due primarily to the weight differences rather than the dietary composition. While no doubt a component of the differences is due to the weight factor, it does not account for the full range of the differences observed.

The average weight of the control animals differs by only 1 g. from the average weight of the fat-rich dieting animals, yet differences in axon diameter between these two groups are significant.

Regardless of causes, the nature of the changes observed requires consideration. Measurements of axon diameters at a given total diameter in the animals on diets free of fat or of protein result in a generally greater axon diameter than in the diets rich in these two components. Inversely it follows, that by subtracting a larger value of axon diameter from the total diameter, the resulting value, which is twice the myelin sheath thickness, is smaller. It therefore seems that if there is a decrease of myelin sheath thickness the axon expands, or if myelin sheath thickness is increased this increase has taken place at the expense of the axon diameter. This problem of course is intimately connected with the question of the qualitative changes associated with the quantitative ones. However, it remains first to be shown, whether a fibre of 5μ total diameter in the protein-rich diet is directly comparable to a fibre of the same diameter in the protein-free one. The indirect evidence on hand at the moment is not sufficient to state whether increase of myelin sheath thickness occurs at the expense of axon diameter or increases the total diameter of a fibre. The evidence available permits both views with qualifications and it is not unlikely that in fact both possibilities are realized at the same time, and perhaps in varying proportions. Recently gained knowledge about the ultrastructure of the myelin sheath and axon would give support also to both these possibilities.

The periodic arrangement of lipids and proteins in the myelin sheath of nerve fibres, as shown indirectly by X-ray diffraction studies, has been thought to result from concentric lamellae of these two components. The use of the electron microscope however, has revealed this lamellation to be in a spiral form (Robertson, 1955 and Finean & Robertson, 1958). Taking these ultra-structural findings into account, there are several ways by which an increase of myelin sheath thickness could come about. As different treatment by fixatives or other reagents produces changes in the width of the periods of these lamellae, as reported by the above-named authors, similarly the quantitative changes observed could be the result of the summation of changes of this kind. The spiral arrangement of the lamellae on the other hand, permits conjecture that these changes result from reduction or increase in the number of periods rather than their width. It is intended to solve this question by direct electron microscopical examination

The 'primum movens' of the changes observed may also be found in the axon itself. Recent studies by Lubinska (1956) and Lubinska & Lukaszewska (1956) have confirmed the views of earlier investigators that the axon is semifluid in nature. Comparatively simple changes in this Sol formation could result in sizeable changes of axon volume. Changes of myelin sheath thickness would then simply be the result of expansion or reduction of the axonal volume.

The endoneural sheath enveloping the outside of the fibres forms a firm stocking-like covering of reticular and collagenous fibres (Plenk, 1934). It is feasible that in the fully developed state this sheath would resist expansion from within; even more so, as examining this sheath with the electron microscope, it could be demonstrated that reticular fibres are not arranged singly but in lamellae or membranes of argyrophilic substance (Tomasch, 1950). It therefore would follow, that myelin

sheath thickness may vary, without the quantity of myelin, that is its phospholipid component, being altered, its layers only becoming more or less compressed.

Studying the effects of protein depletion on the changes associated with degeneration of the myelin sheath in sciatic nerves of rats, Mannell & Rossiter (1954) have assayed the phospholipid content of such nerves under varying conditions. They have demonstrated a correlation between the phospholipid content of the sciatic nerves of rats and their body weight. This single aspect of their findings is sufficient for this discussion as there is proof that quantitative changes in phospholipid content do occur. Therefore, the possibility that the reported differences in myelin sheath thickness are due to changes in the amount of myelin cannot be excluded.

As myelin sheath thickness seems to bear on functional aspects of the nerve fibre, it would be interesting to know whether such differences as reported here result in measurable changes of conduction velocity.

SUMMARY

Measurements were made of the total and axon diameters of nerve fibres. The nerves investigated were the sciatic nerves of rats previously subjected to various diets over a period of 2 months. Differences observed were subjected to statistical reliability tests such as analysis of covariance and the χ^2 test. Comparing effects of a protein-rich and a protein-free diet, or a fat-rich and a fat-free diet, analysis of covariance indicated a significant difference in myelin sheath thickness $P = 0{\cdot}05$.

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THE DEVELOPMENT OF THE HUMAN INTERNAL CAPSULE AND LENTIFORM NUCLEUS

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INTRODUCTION

The head of the human caudate nucleus develops in the floor of the lateral ventricle from three rostro-caudally aligned elevations called medial, lateral and intermediate striatal elevations, successively appearing in that order. Caudally these elevations unite and this part gives rise to the tail of the caudate nucleus and amygdaloid body (Hewitt, 1958).

Accounts of the development of the internal capsule (Arey, 1954; Frazer, 1931; Hamilton, Boyd & Mossman, 1952; Harrison, 1959; Hochstetter, 1929; Johnston, Davies & Davies, 1958; Patten, 1946; and Streeter, 1912) describe its formation from fibres passing to and from the cerebral cortex through a mass of cells, usually called the corpus striatum, in the basal part of the hemisphere. This cellular mass, perhaps better termed Basal Ganglia, forms the substance of the striatal elevations. All but two of the authors to whom reference has been made consider or assume this mass to consist of a homogeneous collection of cells. The exceptions (Hochstetter, 1929, makes no mention in the text of the fact that his illustrations clearly reveal the cells arranged in curved laminae and Hamilton, Boyd & Mossman, 1952) illustrate heavy condensations of cells deep to the surface of the two striatal elevations which these authors in common with all the others mentioned, except one (Harrison, 1959) who describes the three, give as the total number of elevations contributing to the caudate nucleus. All these authors describe how the passage of the internal capsule through the cells of the basal ganglia mass subdivides them into those on its outer side which become the lentiform nucleus and those on the inner side of the internal capsule forming the substance of the caudate nucleus. None of these descriptions fully explains the formation of the parts of the lentiform nucleus, gives adequate details of the stages in the development of the internal capsule or explains the route it follows through the basal ganglia.

MATERIALS

Serial sections, stained with haematoxylin and eosin, of whole embryos and foetuses or their cerebral hemispheres were used. Transverse sections of 7·5 and 25 mm. C.R. length embryos were studied together with a 15 mm. C.R. length embryo which had been orientated so that coronal sections through its cerebral hemispheres were made. Coronal sections of one cerebral hemisphere from embryos and foetuses of 35, 70 and 135 mm. C.R. length were also examined. Except for the sections of the 135 mm. foetus which were $20\,\mu$ thick all the other sections were $10\,\mu$ thick. Wax-

plate reconstructions of the 7·5, 15, 35 and 135 mm. material were also available and these were frequently used to develop a three-dimensional understanding of the sections under examination.

RESULTS

In the 7·5 mm. embryo (Pl. 1, fig. 1) the basal ganglia presented as a single ridge in the side wall of each cerebral vesicle. This ridge becomes the medial striatal elevation in later stages of development (Pl. 1, fig. 1, *M.S.E.*). The wall of the cerebral vesicle consisted of a mantle zone lined on its ventricular surface with an ependymal zone; no marginal zone was visible. The substance of the striatal elevation consisted of a thickening of the mantle zone, composed of loosely arranged cells, continuous with the thinner mantle zone over the rest of the vesicles. The surface of the elevation was covered with the ependymal zone which was much the same thickness as that lining the remainder of the vesicles. There was no sign of an internal capsule.

In the 15 mm. embryo (Pl. 1, fig. 2) the developing head of the caudate nucleus was composed of two contiguous elevations, the medial and lateral striatal elevations (Pl. 1, fig. 2, *M.S.E.*, *L.S.E.*). The medial striatal elevation derived from that seen in the side wall of the cerebral vesicle of the 7·5 mm. embryo now lay in the floor of the hemisphere on the medial side of the second or lateral striatal elevation. Deep to the ependymal zone covering the ventricular surface of these masses was the mantle zone which was considerably thickened to form the substance of the basal ganglia as in the 7·5 mm. embryo. No marginal zone was visible in the region under examination. In the centre of the mantle zone mass, opposite the large interventricular foramen, deep to the surface of the caudal end of the developing head of the caudate nucleus, was a stratified lamina of somewhat more densely packed cells. This lamina, which will be referred to later as the inner or medial lamina of cells, was approximately one-fifth of a millimetre in cranio-caudal extent and was cup-shaped with its mouth directed backwards and medially towards the side wall of the diencephalon (Pl. 1, fig. 2, *M.L.*). Within the concavity of this lamina was a small bundle of fibres (Pl. 1, fig. 2, *I.C.*) which, when followed caudally, passed from the mouth of the lamina to the side wall of the diencephalon through the cerebral peduncle. This bundle of fibres constituted the early formation of the internal capsule.

In the 25 mm. embryo (Pl. 1, fig. 3) apart from the pallium which now possessed a thin cortical layer of densely arranged cells extending down over the upper part of the outer surface of the basal ganglia, these otherwise consisted of the same components as those of the 15 mm. embryo. The internal capsule was thicker than previously observed and principally consisted of parallel upper and lower components directed to the diencephalon but not extending to the pallium. Between these two components was a third, less obvious, intermediate set of fibres interspersed with cells reaching as far as the pallium (Pl. 1, fig. 3, *I.C.*). The upper component of the internal capsule extended between a small, dense, round condensation of cells in the mantle zone and the diencephalon (Pl. 1, fig. 3, *M.L.*). This mass, or medial lamina, was deep to the medial striatal elevation on and behind the plane of the interventricular foramen and its cells were almost continuous with the thick ependymal zone on the ventricular surface of the elevation (Pl. 1, fig. 3, *M.S.E.*).

This lamina had the same cup shape and bore the same relations to the upper part of the internal capsule as the single lamina and internal capsule bore to one another in the 15 mm. embryo and were considered equivalent.

The lower component of the internal capsule emerged just above the lower edge of a second curved lateral lamina of cells lying outside the medial lamina (Pl. 1, fig. 3, *L.L.*). This lamina was deep to the lateral striatal elevation and when traced upwards its cells became continuous with those deep to the ependymal zone on the surface of the striatal elevation (Pl. 1, fig. 3, *L.S.E.*). The curvatures of the two laminae were the same and were disposed along the circumference of concentric circles, except below where the lateral lamina joined a thicker dense mass of cells in the lowest part of the basal ganglia forming a triangular mass the apex of which extended medially below the medial lamina. It was to the upper surface of this mass close to its apex that the lower component of the internal capsule was attached. The intermediate component of the internal capsule extended to the pallium and consisted of fine fibres interspersed with cells of the lower edge of the medial lamina and centre of the lateral lamina through which the fibres passed. Thus the intermediate portion of the internal capsule, although not completely interrupting the continuity of the cells in the laminae, was beginning to trace out the path which other fibres would follow in order to complete the adult appearance of the internal capsule. Below and lateral to the internal capsule the triangular mass of mantle zone cells already mentioned had the form of the adult lentiform nucleus in coronal section (Pl. 1, fig. 3). Thus it was apparent that the developing internal capsule was beginning to split the cells of the two laminae into those joining the caudate nucleus on the ventricular side and those forming the lentiform nucleus on the lower and outer side. Although the parts of the lentiform nucleus could not be distinguished yet, nevertheless, it was now possible to discern how the globus pallidus could be partially or completely derived from the lower part of the medial lamina and the putamen could be formed from the lower part of the lateral lamina (Text-fig. 1).

In the 35 mm. embryo (Pl. 1, figs. 4–8) the internal capsule consisted of a single thick mass of fibres directed from the diencephalon, through the cerebral peduncle, obliquely forwards and laterally in the hemisphere and its course could only be understood by following it through serial sections with the aid of the reconstruction. Caudally the internal capsule (Pl. 1, figs. 4, 5, *I.C.*) was visible as a thick mass of fibres extending through the cerebral peduncle between the side of the diencephalon and the mass of the basal ganglia. The sections transecting this part of the internal capsule were passing through the mantle zone deep to the posterior end of the developing head of the caudate nucleus where its components were almost united into the tail. It was, however, still possible to discern vaguely the two striatal elevations forming the head of the nucleus. Deep to the caudate nucleus the two curved laminae of densely packed cells were even more clearly visible and now obviously continuous with the cells of the striatal elevations deep to which they were each placed as before (Pl. 1, figs. 4, 5, *M.L.*, *L.L.*). The internal capsule extended between the medially directed concavity of the medial lamina to the diencephalon. Below the internal capsule the cells of the medial lamina formed a tongue-like process separated from the apex of the triangular mass, of the lower part of the lateral lamina, by a thin layer of paler-staining loosely packed cells. This tongue-

like process was probably the portion of the medial lamina involved in the formation of the globus pallidus. In addition, a strand of cells was also given off from the lower part of the lateral lamina and joined the tongue of cells from the medial lamina. This may also have been concerned with the formation of the globus pallidus. Proceeding rostrally (Pl. 1, fig. 6) the internal capsule transected both laminae and the lentiform nucleus was apparent and resembled that seen in the 25 mm. embryo. The only other feature was the tongue-like processes from both laminae.

Even further rostrally (Pl. 1, figs. 7, 8) the cells of the mantle zone deep to the developing caudate nucleus were undifferentiated and had no characteristic arrangement. Because of its oblique course, in those sections traversing the anterior end of the forwardly directed internal capsule, only the outer part of the internal capsule was visible just deep to the junctional zone between caudate nucleus and pallium (Pl. 1, figs. 7, 8, *I.C.*). Here the internal capsule had fanned out and was arranged in bands or bundles with intervening strands of cells resembling the adult anterior limb of the internal capsule, where this is similarly split by strands of grey matter extending between the putamen and caudate nucleus.

In the 70 and 135 mm. foetuses the internal capsule, caudate nucleus and lentiform nucleus were similar in form and resembled the adult appearance, and these two foetuses were considered together. In both specimens the third element of the caudate nucleus, the intermediate striatal elevation, had intervened between the other two (Hewitt, 1958). At these stages of development the internal capsule completely separated the cells forming the caudate and lentiform nuclei from one another, except rostrally where they were in continuity over the region of the olfactory tubercle. In earlier stages of development cells of each lamina forming the two nuclei were in continuity and these were able to keep pace with one another in their growth in width before the internal capsule split them. Nevertheless, in spite of the intervention of the internal capsule between the two nuclei in these 70 and 135 mm. foetuses the lentiform nucleus continued to increase in width and had kept pace with the increasing width of the caudate nucleus resulting from the addition of the intermediate striatal elevation. In the 70 mm. foetus the caudate nucleus consisted of a thick deeply staining ependymal layer on its surface and deep to this less densely staining, more loosely packed mantle layer cells as in previous embryos and foetuses. The lentiform nucleus consisted of loosely packed cells similar to those in the deep part of the caudate nucleus. For the most part the globus pallidus was still not distinguishable in this foetus. In almost all sections of this specimen the structure and staining reactions were uniform throughout the lentiform nucleus. In one or two sections through the posterior end of the nucleus, however, its two parts were faintly distinguishable because the staining reaction of the globus pallidus was slightly less intense than the putamen, and between the two a thin line was visible because of the reduced density of the cells. On the other hand the globus pallidus was clearly defined in the 135 mm. foetus (Text-fig. 2). In this foetus the material composing the caudate nucleus deep to the now thick ependymal layer and the putamen of the lentiform nucleus had not the homogeneous appearance of the previous specimen, but consisted of scattered irregular masses of cells intermingled with fibres. The cells comprising the globus pallidus on the other hand had retained

their homogeneous appearance so that this was now clearly distinguishable. In addition, the material comprising the globus pallidus had become more eosinophilic in its staining reaction than the material comprising the putamen and deep part of the caudate nucleus and this provided another distinguishing feature. The pallium in both foetuses was also by now much thicker and more complex in structure, and the internal capsule could easily be traced into the intermediate layer or deeper parts of the mantle zone.

DISCUSSION

Early in the development of the cerebral hemisphere a cup-shaped lamina of cells forms in the mantle zone deep to the surface of the developing head of the caudate nucleus. The mouth of this lamina is directed towards the diencephalon and between these a small bundle of fibres can be traced; this constitutes the beginnings of the internal capsule. At a slightly later stage of development two concentric curved laminae of cells are present in the mantle zone. These lie deep to the medial and lateral striatal elevations, which at this stage of development are the only components present out of the three which ultimately contribute to the formation of the head of the caudate nucleus. The cells of these laminae are in continuity with the cells deep to the striatal elevations; the medial lamina lying deep to the medial striatal elevation and the lateral lamina lying deep to the lateral striatal elevation. The concavities of these laminae are also directed towards the diencephalon. These laminae can be clearly seen in the illustrations of Hochstetter (1929). When these two laminae are first seen the internal capsule consists of three parts. The upper part extends between the medial lamina and the diencephalon. This lamina and this part of the internal capsule are probably equivalent to the single lamina and bundle of fibres first seen. The lower part of the internal capsule extends between the diencephalon and the apical region of the triangular mass of cells into which the lower edge of the lateral lamina thickens. Between these two parts of the developing internal capsule a third set of fine fibres, interspersed with cells, extends between the pallium and diencephalon transecting both laminae. Thus a simple internal capsule has for med and below and lateral to this it is now possible to discern the triangular shape of the lentiform nucleus formed from both laminae. The cells in the striatal elevations on the ventricular side of the internal capsule contribute to the formation of the caudate nucleus (Text-fig. 1). Later by the further addition of fibres the internal capsule becomes a single bundle of fibres transecting the two cellular laminae.

The precise contribution made by the two laminae to the two parts of the lentiform nucleus is not completely clear. Undoubtedly much of the putamen is derived from the lower part of the outer lamina, but whether it contains cells derived from the medial lamina adjacent to it is difficult to determine by the examination of serial sections alone. It is very reasonable to conclude that the globus pallidus is derived from that part of the inner lamina which lies below the site of its transection by the internal capsule, and this lamina being the first of the two to develop would conform with the belief that the globus pallidus, often called the paleostriatum, is phylogenetically the oldest part of the lentiform nucleus. The globus pallidus may, however, also receive a contribution either from the tongue of cells from the lateral lamina or from its lower lip where this curves below the medial lamina (Text-fig. 1

and Pl. 1, figs. 4, 5). The determination of the structures contributing to the globus pallidus might be aided by locating the iron which is said to be present in structures derived from the paleostriatum.

The third and major component of the caudate nucleus or intermediate striatal elevation develops when the internal capsule has completely transected the two laminae except over the region of the olfactory tubercle. Thus it is impossible for a third lamina to develop as a continuous sheet alongside, or between the other two, having continuity with the intermediate striatal elevation and developing lentiform

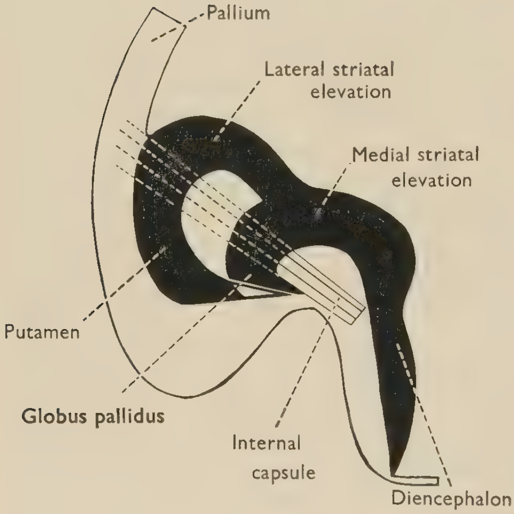


Fig. 1

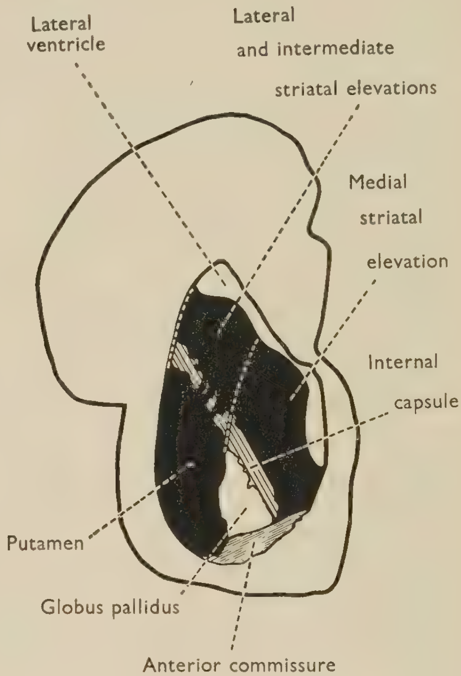


Fig. 2

Text-fig. 1. Diagram of a coronal section through the basal part of an embryonic left cerebral hemisphere. The curved inner and outer laminae of cells in the mantle layer have been made continuous with the medial and lateral striatal elevations contributing to the formation of the head of the caudate nucleus. The internal capsule can be seen extending between the diencephalon and pallium transecting the inner and outer laminae. The uninterrupted lines of the internal capsule represent early stages in its development from the concavity of the medial lamina. The interrupted lines depict extension of the internal capsule laterally to the pallium through both laminae subdividing them. The illustration demonstrates the globus pallidus forming from the lower part of the inner lamina and the derivation of the putamen from the lower part of the outer lamina. The part of the lateral lamina (putamen) marked in white below the inner lamina is to show how this could contribute to the globus pallidus.

Text-fig. 2. Line drawing of coronal section through the left cerebral hemisphere of a 135 mm. C.R. length foetus. The caudate nucleus consists of three striatal elevations. The lentiform nucleus is divided into putamen and globus pallidus. Note the section has transected the anterior commissure where it lies immediately below the lentiform nucleus. The dotted lines have been drawn upwards from the inner and outer borders of the putamen following its curvature. The lateral and intermediate striatal elevations of the caudate nucleus lie between these lines suggesting that the putamen is equivalent to these parts of the caudate nucleus.

nucleus such as existed with the first two laminae before their transection by the internal capsule. Nevertheless, the caudate and lentiform nucleus keep pace with one another in their subsequent increase in transverse width. This can be shown in a coronal section of a fully differentiated or adult lentiform nucleus by drawing two lines upwards from the inner and outer borders of the putamen following their curvatures, as in Text-fig. 2, and it will be found that these intersect the caudate nucleus at the junction of its medial and intermediate striatal elevations and its outer edge, respectively. Thus it may be concluded that the putamen increases in width by the addition of further cells at the same time as the caudate nucleus broadens after the intermediate striatal elevation appears. This occurs even though a third continuous lamina cannot be formed between the putamen and intermediate striatal elevation because by this time the internal capsule is intervening.

The orientation of the medial lamina and the direction of its mouth determines the path of the internal capsule. The internal capsule may be likened to a bullet fired from a rifle. The path it follows is governed by the line of aim or direction in which the rifle is pointing. When the internal capsule cuts through the lamina it follows the line of the fibres emerging from the mouth of the lamina just as the bullet, if it could fire backwards through the breech, would follow the same line of aim as before. The internal capsule joins the pallium at its point of junction with the lateral edge of the caudate nucleus, but it could pass through the basal ganglia in any direction to join the pallium below this point. It could not, however, join the pallium at a higher point, otherwise it would have to pass through the cavity of the ventricle. No reason can be advanced, however, to explain why the internal capsule does not traverse the basal ganglia in a different line or even pass below them to join the pallium at a lower point.

When the fibres forming the internal capsule first begin to intersect the cells of the laminae the fibres and cells are intermingled; the cells predominating. As further fibres are added to the internal capsule the cells diminish in number until they disappear except in the anterior limb of the internal capsule where they remain as strands connecting the putamen and caudate nucleus and at the extreme anterior end over the olfactory tubercle where the two nuclei are completely fused. This state of affairs is first seen in the 35 mm. C.R. length embryo (Pl. 1, fig. 7). From these facts it would be reasonable to deduce that the fibres of the internal capsule are being built up from behind forwards so that in the anterior limb of the internal capsule the number of fibres present is insufficient to completely obliterate the strands of grey matter connecting the caudate nucleus and putamen. The part of the caudate nucleus fused with the putamen over the olfactory tubercle could reasonably be expected to occur with that part of the caudate nucleus derived from the lateral and intermediate striatal elevations, if the deductions discussed and illustrated in Tex-fig. 2, concerning the corresponding parts of the putamen and caudate nucleus, are correct. A number of efforts to demonstrate this have been made but they have always implicated the medial striatal elevation in the fusion. One possible explanation for this is that the globus pallidus is derived from the central portion of the medial lamina of cells and the lower end of this contributes to part of the putamen.

SUMMARY

1. The development of the human internal capsule and lentiform nucleus has been studied using serial coronal sections of the heads or cerebral hemispheres of embryos and foetuses of C.R. lengths ranging from 7·5 to 135 mm.

2. In the mantle zone deep to the developing caudate nucleus in a 15 mm. C.R. length embryo a cup-shaped lamina of densely grouped cells appears with its concavity facing towards the diencephalon. Later in the 25 mm. C.R. length embryo another dense collection of cells in the mantle zone forms a second curved lamina lateral to the first and both laminae become continuous with the cells deep to their respective striatal elevations.

3. The internal capsule first appears as a small bundle of fibres in the concavity of the first or medial lamina of cells in the 15 mm. embryo. These can be followed between the lamina and diencephalon. In the 25 mm. embryo the medial lamina becomes larger and the fibres from it increase in length and number. A second bundle of fibres also develop from the lower edge of the lateral lamina. These two bundles are parallel, directed towards the diencephalon and between them the cells of both laminae are less densely arranged. Among these cells fine fibres can be seen extending to the pallium. In the 35 mm. embryo all three parts of the internal capsule seen in the last embryo have united into a single thick bundle directed obliquely between the diencephalon and the pallium splitting both laminae of cells.

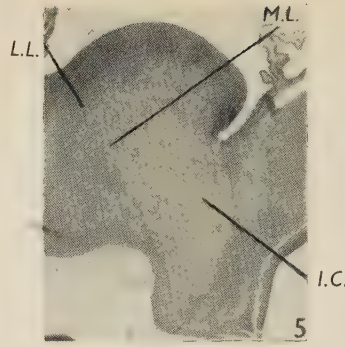
4. When the internal capsule has split the laminae the parts of these deep to the striatal elevations contribute to the formation of the caudate nucleus. The part of the medial lamina below the internal capsule forms much if not all of the globus pallidus, although it may contribute to the putamen where it lies adjacent to the lateral lamina. The part of the lateral lamina deep to the internal capsule forms the putamen but it is possible that it contributes to the globus pallidus also.

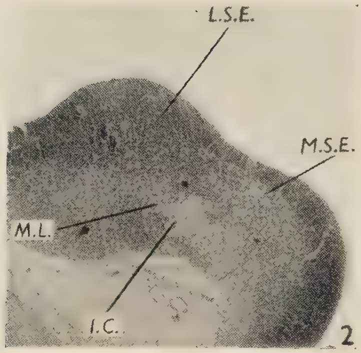
5. The 70 and 135 mm. foetuses in many ways resemble the adult appearance. The globus pallidus is faintly visible in the 70 mm. foetus and clearly distinguishable in the 135 mm. foetus. In both these foetuses the third or intermediate striatal elevation has commenced developing in the caudate nucleus, and although the internal capsule is separating the caudate nucleus from the lentiform the width of the latter keeps pace with the increasing width of the caudate nucleus. It appears that the putamen is the part of the lentiform nucleus which corresponds to that part of the caudate nucleus derived from the lateral and intermediate striatal elevations.

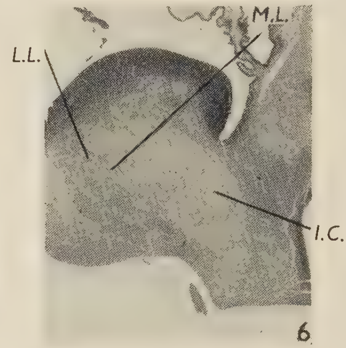
6. The direction of the internal capsule is discussed and is probably originally determined by the orientation of the medial lamina when this is first formed. The internal capsule forms along an axis at right angles to the centre of the mouth of this lamina.

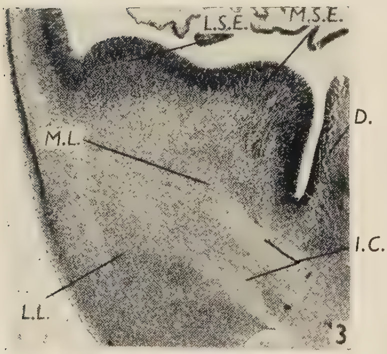
7. The parts of the developing laminae involved in the fusion between the front end of the putamen and the head of the caudate nucleus are discussed but no conclusions are reached.

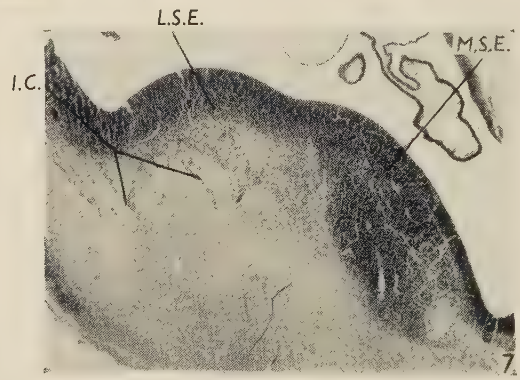
My thanks are due to Mr A. V. Freeborn for his technical assistance and to Mr J. S. Fenton for carrying out the photography.

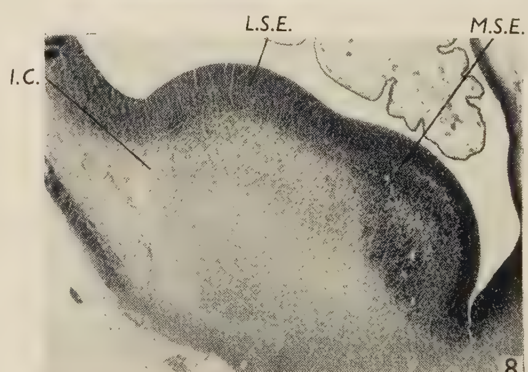












HEWITT—The human internal capsule and lentiform nucleus

(*Facing p.* 199)

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EXPLANATION OF PLATE

Coronal sections through the cerebral hemispheres of human embryos. Except Fig. 1, all are sections of left cerebral hemispheres. Figs. 2–8 only include the region of the basal ganglia. All sections have been stained with haematoxylin and eosin and are 10μ thick.

Fig. 1. Cerebral vesicles of a 7·5 mm. c.r. length embryo showing striatal elevations in each side wall composed of an undifferentiated mantle zone deep to the darker staining ependymal zone. ×25.

Fig. 2. 15 mm. c.r. length embryo. In the mantle zone is a small curved lamina of cells deep to the groove between the medial and lateral striatal elevations. In the concavity of the lamina, which is directed medially, lies a small collection of fibres, the early form of the internal capsule. ×30.

Fig. 3. 25 mm. c.r. length embryo. The internal capsule is composed of three parts. The upper part is emerging from the centre of a dense mass of mantle zone cells, the medial lamina, deep to the medial striatal elevation. The lower part of the internal capsule is somewhat smaller than the upper part and is continuous with the lateral lamina of cells lateral to the medial lamina. The lower part of this lateral lamina is most conspicuous by its triangular shape and has the form of the lentiform nucleus. When followed up this lamina becomes continuous with cells deep to the lateral striatal elevation. Between the two parts of the internal capsule, and in line with these but extending further laterally, the cells are much less densely arranged because intermingled with them are the fine fibres of the third part of the internal capsule extending to the pallium. These fibres cannot be seen in the photograph. Note also the cortical layer in the pallium extending over the surface of the upper part of the basal ganglia. ×25.

Fig. 4. 35 mm. c.r. length embryo. This section is through the posterior end of the internal capsule, a solid mass of fibres in the cerebral peduncle proceeding obliquely forwards as far as the medial lamina of cells. Note the tongue of cells from the lower edge of the medial lamina and another strand of cells from the lateral lamina joining the lower part of the medial lamina. ×10.

Fig. 5. Same embryo as Fig. 4, but further rostrally. The internal capsule is just beginning to extend through the medial lamina of cells. ×10.

Fig. 6. Same embryo as Figs. 4 and 5 but further rostrally. The internal capsule is beginning to intersect both laminae. ×10.

Fig. 7. Same embryo as Figs. 4, 5 and 6 but further rostrally. The internal capsule has reached the pallium and consists of bundles of fibres separated by bands of cells similar to the appearance of the adult anterior limb of the internal capsule. ×25.

Fig. 8. Same embryo as Figs. 4 to 7. This shows the most rostral end of the internal capsule which is small but in a similar position to that in Fig. 7. ×25.

ABBREVIATIONS

D.	Diencephalon	*L.L.*	Lateral lamina of cells	*M.L.*	Medial lamina of cells
I.C.	Internal capsule	*L.S.E.*	Lateral striatal elevation	*M.S.E.*	Medial striatal elevation

OBSERVATIONS ON THE FULL-TERM FOETAL MEMBRANES OF THREE MEMBERS OF THE CAMELIDAE (*CAMELUS DROMEDARIUS* L., *CAMELUS BACTRIANUS* L. AND *LAMA GLAMA* L.)

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Our knowledge of the placentae in the Camelidae is largely based on the macroscopic examination, made many years ago, of a small number of specimens, and on more recent unillustrated statements of other workers. Savi (1843) was the first to note that the foetal placenta in the dromedary was diffuse in nature, as in the mare, and not cotyledonary, as in other ruminants. He described the thick chorionic membrane in the dromedary as being covered with short shrub-like tendrils or villi, in which arteriovenous connexions sometimes occurred. He considered, on these and other taxonomic points, that camels were more related to Pachyderms than to Ruminants. Owen (1868), Milne-Edwards (1870), Turner (1875), and Beddard (1902) have noted the diffuse nature of the placenta in the Camelidae, and Turner also recorded the presence of polar bare areas. Grosser (1927) and Mossman (1937), apparently basing their opinions on the accounts of Owen and Beddard, classified the placenta in the Bactrian camel as undoubtedly epithelio-chorial in type. Barmintsev (1938, 1939), and Boshaev (1938) gave tables on the gestation period, mating and oestrous cycles for both the Arabian (*Camelus dromedarius* L.) and the Bactrian camel but did not describe their foetal membranes. Barclay, Franklin & Pritchard (1944) figured a transverse section of the umbilical cord of a dromedary foetus of unknown age, illustrating the allantoic duct, the remains of the yolk-sac, and amniotic pustules; and Amoroso (1952), and Harvey (1959), referred to, but did not illustrate, the epithelio-chorial nature of the placenta of camels. In the absence of recent descriptions of the membranes a description of the full-term foetal membranes of three members of the Camelidae, together with illustrations of their macro-, and microscopic appearances is of interest.

MATERIALS AND METHODS

The material on which the present account is based consisted of the foetal membranes obtained immediately after delivery from an Arabian camel (*C. dromedarius* L.) following a gestation period of 417 days from the last day of mating; and those from a Bactarian camel (*C. bactrianus* L.) and a llama (*Lama glama* L.) obtained about 24 hr. after delivery of normal full-term calves. All three calves were singleton females, and all thrived after birth. The specimens of Bactrian and llama placentae were damaged after delivery, but the general form and histological appearances could be determined.

Small portions of the dromedary chorion, and all that part of the amnion which was born with the calf, were fixed in 10% formol-saline, or absolute alcohol, immediately after the birth of the calf and membranes. Further samples of the chorion, amnion and allantois were taken after the membranes had been examined and photographed, and fixed in 10% formol-saline some 2 hr. after delivery. The two other specimens were cleansed with water and photographed before similar samples were removed and fixed in 10% formol-saline or Bouin's solution. Specimens from the three animals were dehydrated, embedded in paraffin, cut at 6, 8, or 10 μ, and stained with Harris's haematoxylin and eosin, Weigert's iron haematoxylin and van Gieson's connective tissue stain, Mallory's or Masson's trichrome stain, Feulgen and Wilder's silver impregnation methods. Frozen sections of formalin fixed material were stained for lipides with Sudan IV. A dye coupling method (Burstone, 1958) was used to demonstrate the presence of alkaline phosphatase.

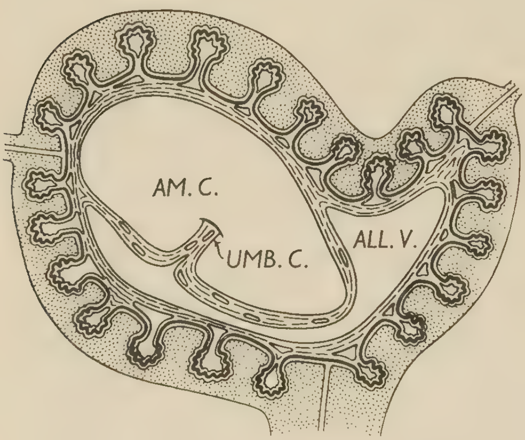
DESCRIPTION

The Arabian camel (Camelus dromedarius *L.*). *Macroscopic and microscopic appearances*

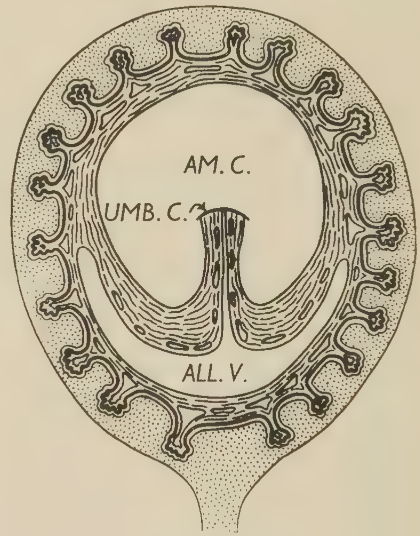
The membranes externally were bright red in colour during delivery, but smooth white inner structures were visible through tears in the chorion. The chorion formed a crescentic sac (Pl. 1, fig. 1) ruptured irregularly near the mid-point of its lesser curve. It measured 10 ft. 7 in. along the greater and 8 ft. 3 in. along the lesser curve when spread out flat on the ground. The two horns of the sac were of unequal size, the larger measuring 20 and 38 in. circumferentially, and the smaller 19 and 28 in. at distances of 1 ft and 3 ft. respectively from the apices. The umbilical cord after rupture measured about 5 in. from the navel to its free end, and another 12 in. to its rather indefinite ending on the membranes. The amnion covered most of this part of the cord. Two umbilical arteries and two veins were present in the cord, and distally were distributed over the whole inner surface of the chorion, each main artery or vein being distributed in general to one half of the chorion. The outer surface of the chorion was covered with small red tufted villous processes (Pl. 1, fig. 2) which varied in height and size, and also in density of distribution. Several areas, mainly near the antimesometric aspect of the larger horn, and near the pole of the lesser horn, were relatively bare of villous tufts, but under a low-power magnifier small sessile elevations could be seen scattered sparsely over these areas. In other areas relatively small tufts were surrounded by larger ones, but typical areolae such as those of the horse or pig, were not seen. The chorionic tufts measured up to 3 mm. in height, and were attached by constricted stalks to the chorion. Distally the surface of each tuft was plicated into numerous folds and did not form finger-like villi such as are seen in the mare's placenta. Small white masses were also present on the edges of some folds. The amnion, most of which had been delivered with the calf, was adherent in part to the chorion forming a chorio-amnion; it also formed an allanto-amnion which was lightly vascularized near the umbilical region (Pl. 2, fig. 7). Small amniotic pustules, measuring up to 1 cm. in diameter, and fine bristle-like horns up to 15 mm. in length were present, being most noticeable close to the umbilical cord (Pl. 2, figs. 8, 9). The allantois, which also was torn,

excluded the amnion almost completely from the lesser horn and from the lesser curve of the chorionic sac where the main branches of the umbilical vessels lay. A soft brown lamellated hippomane measuring 65 × 32 × 17 mm. and weighing 18 g. was found in the allantoic cavity.

Low-power microscopical examination of the external surface of the chorion, and sections transverse and tangential to its surface demonstrated clearly that the villous tufts were folded and not branched (Pl. 1, fig. 2; Pl. 3, figs. 15, 16). The plicated villous tufts were quite different in appearance from the fine finger-like processes radiating from a central core which are found in the placentae of the mare, pony and donkey. They consisted of a central mesenchymal core covered by a thin layer of trophoblast, the cell walls of which were difficult to define. Large masses of syncytiotrophoblast were also present, and frequently projected from the apices of



Text-fig. 1



Text-fig. 2

Text-fig. 1. Diagrammatic representation of the probable arrangement of the foetal membranes of the Camelidae at full term. Compound crypts in the uterine wall (stippled) lined by intact uterine epithelium enclose the diffusely scattered plicated villous tufts of the chorion forming a diffuse epithelio-chorial placenta. The relatively small allantoic vesicle excludes the amnion from the smaller pole of the chorion, but the amnion obliterates the allantoic cavity in large part, and may form a true amnio-chorion.

Text-fig. 2. Schematic diagram of a transverse section through the pregnant horn of the uterus. Abbreviations: *ALL.V.*, allantoic vesicle; *AM.C.*, amniotic cavity; *UMB.C.*, umbilical cord.

the villous folds. These masses may represent the white structures visible on surface inspection of the chorion. Areas of well defined tall columnar-celled cytotrophoblast were also present (Pl. 3, fig. 17) basally and may represent the normal cryptal trophoblast, such as is seen in the pig (Amoroso, 1955), or specialized areas for direct absorption of material from the uterine lumen as described by Hamilton, Harrison & Young (1960) in the Cervidae. A rich interlacing network of capillary vessels was present superficially, which on section was found to be intra-epithelial in position

(Pl. 3, figs. 18–20). A subepithelial plexus was also present, and the larger vessels lying looped in the mesenchymal core were connected to the plexuses at frequent intervals. Intra-epithelial capillaries were absent from the areas of columnar cytotrophoblast. A short piece of the umbilical cord, fixed between ligatures while still distended with blood, measured $2{\cdot}5 \times 1{\cdot}8$ cm. in cross-section and contained two umbilical arteries, two umbilical veins, the patent remains of the allantoic duct, and a number of small vasa propria embedded in loose mesenchyme and covered by the amnion (Pl. 2, fig. 12). The umbilical veins were slightly larger than the arteries and resembled them in having muscular walls interlaced by elastic tissue and a lining of low cuboidal endothelium. The allantoic duct was lined by tall columnar cells, which rested on a basal layer of small cuboidal cells. External to this was a connective tissue layer 0·13–0·2 mm. thick, and then a layer of longitudinally running smooth muscle some 0·4–0·5 mm. thick. Capillary vessels at right angles to the long axis of the duct penetrated the connective tissue zone as far as the epithelium. The remains of a small highly vascular yolk-sac were found under the amniotic covering of another part of the umbilical cord. It was lined with cuboidal cells which in many places were heaped up into projecting masses. The vitelline vessels ran longitudinally along the main axis of the sac. The amnion was lined with a single layer of flattened epithelial cells which formed low cornified plaques or became columnar in the larger pustules. Foci of cell degeneration were present in the latter, and occasionally epithelial 'pearls' were formed. The allantoic hippomane consisted of a mass of degenerated cells, some giant cells with well-stained nuclear masses, small intact groups of epithelial cells, and a few small cysts, all bound together by layers of fibrinous material. The probable *in situ* relationships of the foetal membranes and the uterus are represented diagrammatically in Text-figs. 1 and 2.

The Bactrian camel (Camelus bactrianus *L.*) *and the llama* (Lama glama *L.*)

The foetal membranes of the Bactrian camel (Pl. 1, figs. 3, 4) and of the llama (Pl. 1, figs. 5, 6) resembled those of the Arabian camel (*C. dromedarius* L.) in their general form and appearances, but they were smaller and less bulky. The fresh weights of the present specimens were not recorded, but that of the dromedary was distinctly heavier than that of the Bactrian camel. The dromedary chorion felt slightly shicker than that of the Bactrian camel. The llama membranes were light and fine as compared with those of the camels. The measurements of the present specimens are compared in Table 1 with those of a Bactrian camel and a llama from the London Zoological Gardens made by Hill (1959). There is a general agreement on the order of the measurements except for the length of the greater curve of the Bactrian chorion. Differences in placing the membranes for measurement, and damage to the membranes probably account for this discrepancy. It will be seen that Hill also noted areas of dense villous concentrations in addition to bare areas on the surface of the chorions. He did not record the presence of hippomanes. The bare areas of the present Bactrian chorion were situated over part of the greater curvature of the intact horn (the other horn was badly damaged), but in the llama a well defined bare area about 3 cm. wide ran along the lesser curve from one pole of the chorion almost to the other, in addition to the areas of relative bareness at the smaller pole and over the greater curve and side walls of the chorion. The linear

bare area (Pl. 1, fig. 6) was related to the underlying main chorionic blood vessels which ran towards the poles from the slightly eccentrically placed umbilical cord. Definite polar bare areas were not seen at all six cornua, nor were bare areas seen which could be related to the probable positions of the internal os uteri. The vascularity of the bare areas in all three specimens was relatively poor as compared with that of areas of dense villous concentration. These latter areas were particularly noticeable on the llama chorion, where small circular areas devoid of villi were in some places surrounded by large tufts resembling areolae (Pl. 2, fig. 11). A somewhat similar condition was also found on parts of the Bactrian chorion (Pl. 2, fig. 10).

Table 1. *Measurements and other data on the foetal membranes of the present specimens compared with those on two specimens from London made by Dr W. C. O. Hill (personal communication)*

	Present specimens			London specimens	
	Arabian camel	Bactrian camel	Llama	Bactrian camel	Llama
Chorion					
Length of lesser curve (in.)	99	68	48	75	$49\frac{1}{2}$
Length of greater curve (in.)	127	89	86	148	78
Maximum girth (in.)	38	49	24	50	21
Length of umbilical cord (in.)	18	—	—	27	—
Bare areas on chorion	Yes	Yes	Yes	Yes	Yes
Heavy villous concentrations on chorion	Yes	Yes	Yes	—	Yes
Hippomanes	Yes	—	Yes	—	—

The macro- and microscopic appearances of the villous tufts of the Bactrian camel (Pl. 4, figs. 21–26) and of the llama (Pl. 5, figs. 27–32) were very similar to those of the dromedary (Pl. 3, figs. 15–20). The tufts were plicated, highly vascular with intra-epithelial capillaries in areas of low trophoblast, had areas of tall columnar-celled cytotrophoblast basally, and masses of syncytiotrophoblast elsewhere. Amniotic pustles were also present, and the allantoic lining, particularly in the llama, was ridged internally (Pl. 5, figs. 28, 29). A soft brown hippomane measuring $38 \times 25 \times 12$ mm. was found in the amniotic cavity of the llama (Pl. 2, fig. 14). It had the same laminated structure as that of the dromedary, and similar microscopial appearances on section.

Special staining techniques. All three placentae showed fine red granules of lipide material scattered throughout the epithelial cells and underlying mesenchyme of the chorion, when stained with Sudan IV. The phosphatase reaction (in dromedary and llama only) produced a marked red stain in the coagulum superficial to the trophoblast, but phosphatase could be detected within any cell layer. A heavy reticulin response was produced by the vessel walls by Wilder's silver impregnation method, and the capillary plexuses were well demonstrated.

DISCUSSION

The mascoscopic appearances of all three placentae are essentially similar, and the arrangement of the amniotic and allantoic membranes also appears to be the same in the three forms. The presence of the diffusely scattered plicated villous tufts

arranged in areas of varying density of distribution is also similar, except that in the llama a more definite bare area was present along the lesser curve of the chorion than was seen in the other two forms. The relative paucity of small blood vessels in the 'bare' or almost 'bare' areas agrees with the observations of Wislocki (1933) who found that the avillous areas of the mare's placenta were associated with relatively avascular zones of the chorion. Mossman (1937), however, considered that such avillous areas were due to the chorion failing to make contact with the uterine mucosa over these areas. The two views do not seem to be mutually exclusive. Hellegers (1960) and Meschia, Prystowsky, Hellegers, Huckabee, Metcalfe & Barron, (1960) have examined llama placentae *in situ* and found that they were epithelio-chorial in type. These investigators deny the view expressed by Prystowsky (1960) that the llama placenta is haemo-endothelial in type. The microscopical findings of the present investigation confirm the presence of the trophoblast layer of the llama chorion. The intact trophoblast with intra-epithelial foetal capillaries, basally situated columnar cytotrophoblast, and syncytiotrophoblast masses elsewhere, as seen in the three camelid placentae, are so similar in appearance to those of the pig and horse at full-term, that it is virtually certain that all three placentae are epithelio-chorial in type. The observations of Turner (1876) indicate that the inter-cotyledonary villi in the giraffe placenta are similar in general morphology to those of the Camelidae, and Mossman (1937) described diffuse patches of small villi between the cotyledons in the chorio-allantoic placenta of the giraffe. It is evident then that the giraffe placenta occupies an intermediate position in its morphology between the pure diffuse placenta of the Camelidae, and of the small musk deer (Owen, 1866–68), and the pure cotyledonary placenta of most ruminants. The observations of Savi (1843) on the foetal placenta of the dromedary have been fully confirmed, and it is shown that his description of the diffusely distributed shrub-like villi could also be applied to the villous tufts of the Bactrian camel and the llama. The great differences between these placentae and those of other ruminants must be taken into consideration as Savi did, when considering the taxonomic position of the Camelidae, and it is surprising to find that this had been done so many years before Mossman (1937) wrote his classical paper. The presence of laminated deposits in the allantois of the dromedary similar to those found in the mare had been noted by Owen (1866–68). Amoroso (1952) records that these bodies occur in the amniotic or allantoic fluids of the horse, cow, sheep and pig. and the present author has found them in the amniotic fluid of the goat (*Capra hircus* L.). The presence of such a body in the amniotic cavity of the llama had not previously been reported. The intra-epithelial position of the capillary network in the chorion and the loops of larger vessels in the cores of the villous tufts which supply the surface vessels are so arranged that 'counterflow' of the foetal and maternal blood streams could take place. Such a 'counterflow', together with the thinness of the tissue layer separating the foetal blood stream from the surface of the chorion, would make the placenta a very efficient organ for foetal-maternal exchanges.

Note. Since going to press the author's attention has been drawn to the article by A. N. Fahmy and M. T. El-Garby (*Gaz. Egypt Soc. Gynaec. Obstet.*, 1959, 9/1) describing the dromedary placenta as non-cotyledonous, epithelio-chorial and with intra-epithelial capillaries.

SUMMARY

1. The full-term foetal chorions of the dromedary, Bactrian camel and llama have been described.

2. The chorion of each is crescentic in shape and diffusely covered by plicated villous tufts, except in irregularly arranged bare areas which may be polar in position.

3. The villous tufts have constricted bases, and bunched and folded extremities. A thin layer of trophoblast containing a profuse intra-epithelial capillary network covers the chorion, except in some basal areas where tall columnar cytotrophoblast is present. Small syncytiotrophoblastic masses are scattered over the surface of the chorion.

4. The allantoic vesicles are relatively small as compared with the amniotic vesicles but in the dromedary excludes the amnion from the distal end of the smaller chorionic cornua.

5. The amnion is large and forms an allanto-amnion which may be partially vascularized. An amnio-chorion appears to be present in the dromedary and probably also in the other species. Small flat amniotic pustules with occasional fine hair-like horns are present in the dromedary. The pustules in the other forms were less conspicuous and without horns.

6. The umbilical cords contained two arteries, two veins, the remains of the allantoic duct and numerous vasa propria.

7. A small highly vascular yolk-sac vesicle was found in the amniotic part of the umbilical cord of the dromedary only.

8. An allantoic hippomane was found in the dromedary and an amniotic hippomane in the llama.

9. The microscopic appearances of the three chorions indicate that the placenta in all three forms is epithelio-chorial in nature.

10. It is suggested that the anatomical arrangement of the chorionic villous vessels is such that 'counterflow' between the foetal and maternal blood streams could occur, and that the placenta, at term, is a very efficient organ for foetal-maternal exchanges.

The author wishes to express his thanks to Mr Alec McLean, M.R.C.V.S., City Veterinarian, Belfast, and Mr R. Chipperfield of Chipperfields Circus and Zoo Ltd., for their great help in obtaining the material on which this research was carried out; to Mr G. Bryans for his technical assistance, and to the Northern Ireland Hospitals Authority for a grant towards the cost of the photography and Queen's University, Belfast for a grant towards the cost of publication.

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EXPLANATION OF PLATES

Plate 1

The gross appearances of the foetal membranes of the Arabian camel (*C. dromedarius* L.), the Bactrian camel (*C. bactrianus* L.), and the llama (*Lama glama* L.) are shown on the left of the plate, while close-up views of the surfaces of the three forms are shown on the right. All illustrations have been taken from Kodachrome transparencies made before the membranes were fixed.

Fig. 1. The foetal membranes of a full-term female calf of the Arabian camel (*C. dromedarius* L.). The two horns of the crescentic chorion are close together at the top left corner of the picture, and the umbilical cord and amnion appear as white structures at the lower right corner, as they protrude through the hole in the chorion caused by the birth of the calf. A 12 in. rule is shown. $\times \frac{1}{14}$ approx.

Fig. 2. Surface view of the dromedary chorion showing the closely spaced plicated placental tufts. $\times 7$ approx.

Fig. 3. The foetal membranes of a full-term female calf of the Bactrian camel (*C. bactrianus* L.). The crescentic form of the membranes can be seen although the chorion has been extensively torn. The umbilical cord above, which is about 18 in. long, leads down to the torn amnion which appears as a bright white structure (lower left), and the inner aspect of the allanto-chorion (lower right). The external surface of the chorion is shown on either side of the umbilical cord. A 12 in. rule is shown. $\times \frac{1}{14}$ approx.

Fig. 4. A low-power view of the surface of the chorion of the Bactrian camel showing the closely packed plicated placental tufts. Scale in mm. $\times 0{\cdot}9$.

Fig. 5. The foetal membranes of a full-term female llama calf (*Lama glama* L.). The crescentic form of the membranes is well illustrated here, as also is the inequality of the two cornuae of the chorion. The umbilical cord, which here is about 6 in. long, leads down to the amnion (left centre) to the left of which large branches of the umbilical vessels can be see on the inner aspect of the allanto-chorion. The narrow white area on the right side of the lesser curve of the chorion is devoid of villous tufts. A 12 in. rule is shown. $\times \frac{1}{12}$.

Fig. 6. Surface view of the bare area on the lesser curve of the llama chorion. There is an absence of the plicated chorionic tufts over the main placental vessels which lie under the white lines in the centre of the picture. The tufts increase gradually in size as the distance from the bare area increases. Scale in mm. ×1·1.

PLATE 2

Figures numbered 9, 10, 11, 13 and 14 are from Kodachrome transparencies made while the membranes were fresh.

Fig. 7. The allanto-amnion of the dromedary by transmitted light showing part of the vascular network, present near the umbilical cord. ×1·3.

Fig. 8. Transverse section of a typical amniotic pustule from the dromedary. ×15.

Fig. 9. The amnion of the dromedary in the umbilical cord region has numerous low ectodermal plaques or pustules on its surface. Scale in mm. ×1·5.

Fig. 10. Surface view of the Bactrian chorion showing areas of large densely clustered villous tufts and other lighter areas of small tufts. ×0·7.

Fig. 11. The chorion of the llama viewed by transmitted light. The villi in the darker areas are large and closely packed while those in the light area are small and few in number. ×3·3 approx.

Fig. 12. Transverse section of the umbilical cord of the dromedary showing two arteries above, and a patent allantoic duct between two umbilical veins below. Numerous small vasa propria are also present. ×3·3.

Fig. 13. Profile view of the chorionic tufts of the llama chorion showing their plicated nature. ×10 approx.

Fig. 14. The amniotic cavity of the llama was found to contain a dark flattened ovoid hippomane which is seen in the centre of the picture partially covered by the cut edge of the allanto-amnion. Scale in mm. ×0·5.

PLATE 3

Photomicrographs of the full-term foetal placenta of the Arabian camel (*C. dromedarius* L.).

Fig. 15. Low-power view of a transverse section through the allanto-chorion of the dromedary. Part of the narrow base of a chorionic villous tuft is seen attached to the chorionic plate, but the majority of the tufts have been cut peripherally. The plicated nature of the tufts is clearly illustrated. The allantois shows as a faint marginal line below. Higher power views of the areas enclosed by the rectangles are shown in figs. 17 and 19. ×29.

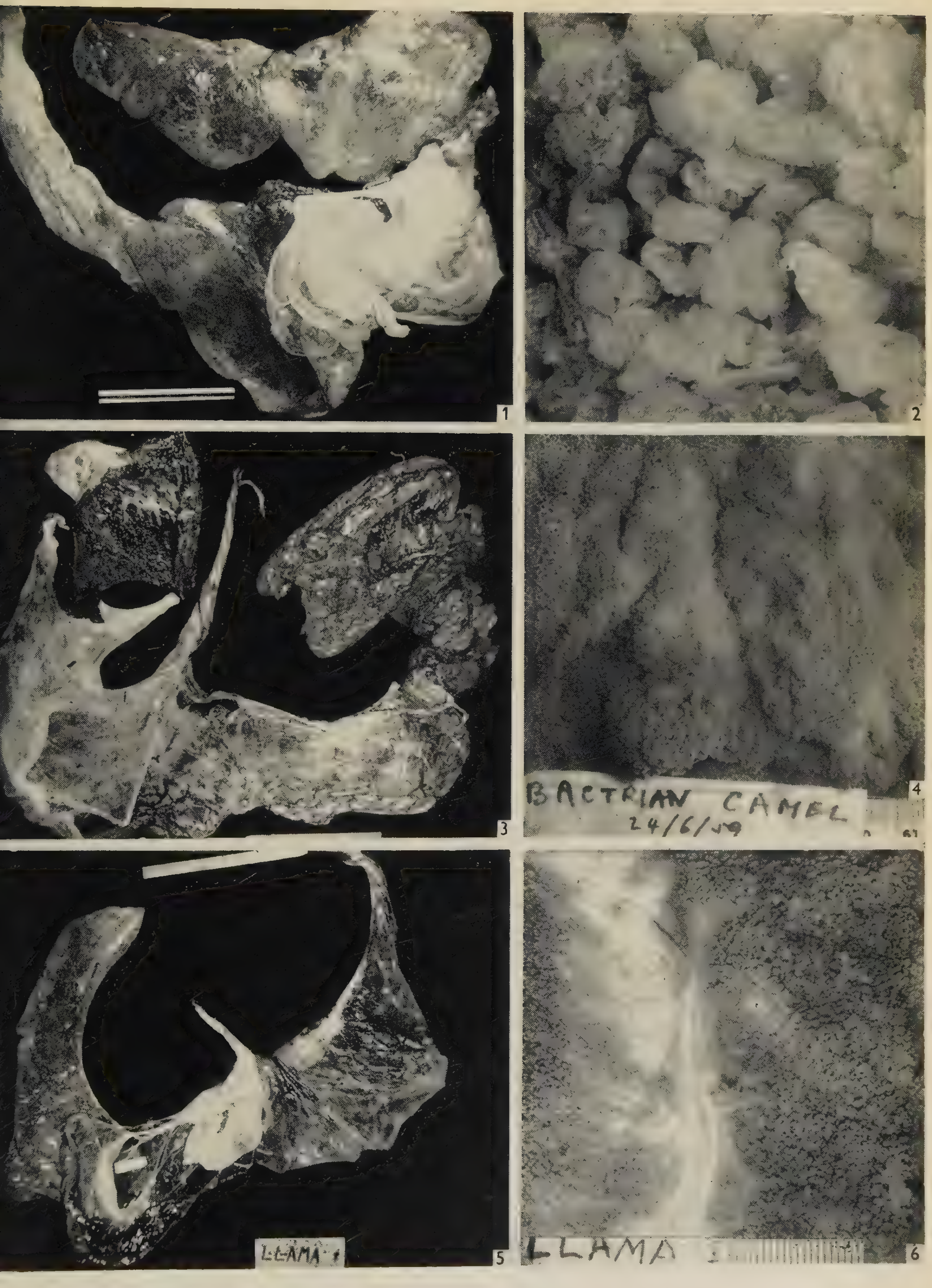
Fig. 16. Tangential section through the chorionic tufts of the dromedary placenta. The star-shaped appearances of the tufts as seen in this view confirm that the tufts are plicated. ×29.

Fig. 17. Medium-power view of the area in the lower right rectangle of Fig. 15. Areas of columnar cytotrophoblast are situated basally in the region of the chorionic plate. ×140.

Fig. 18. High-power view of T.S. of the dromedary chorion showing a large hour-glass shaped intra-epithelial foetal capillary within the trophoblast layer. ×1400.

Fig. 19. Medium-power view of the upper left rectangle of fig. 15. The peripheral trophoblast is low, the cell walls are indistinct, and many intra-epithelial capillaries are present in it. A capillary is seen running from the mesenchymal core of the tuft to the edge of the tuft. A high-power view of this vessel is shown in fig. 20 below. Syncytial masses of trophoblast are also present. ×140.

Fig. 20. High-power view of the area in the rectangle in fig. 19. The foetal capillary is running diagonally up and to the right where it divides into two as it enters the trophoblast layer. Elongated oval red blood cells can be seen within its lumen. ×1400.



MORTON—OBSERVATIONS ON THE FULL-TERM FOETAL MEMBRANES OF CAMELIDAE

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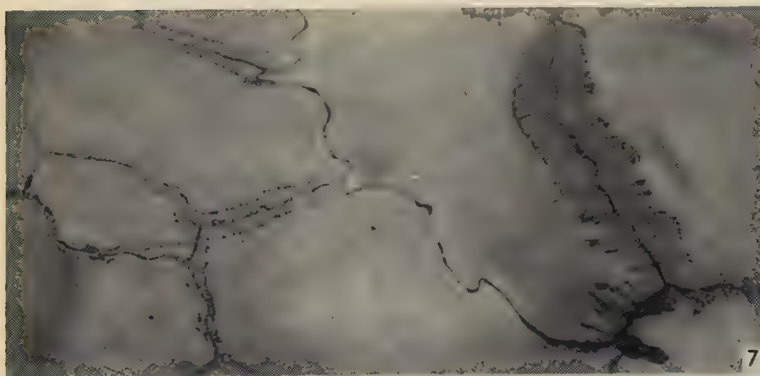
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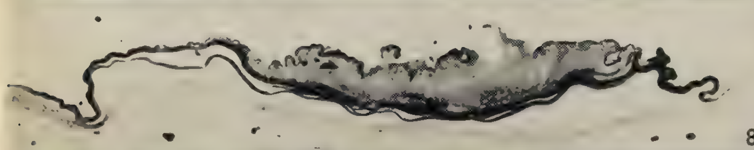
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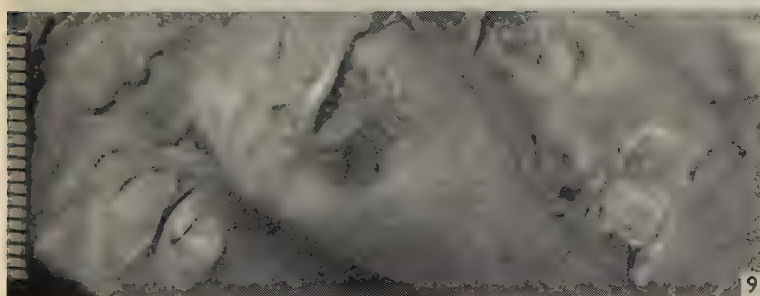
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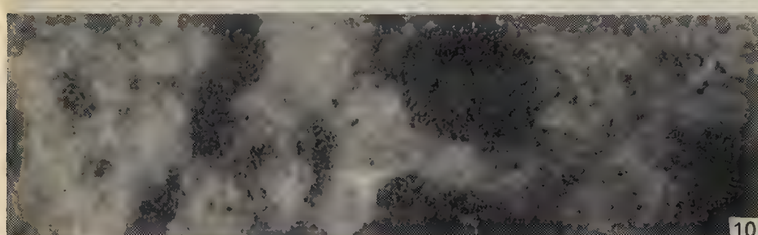
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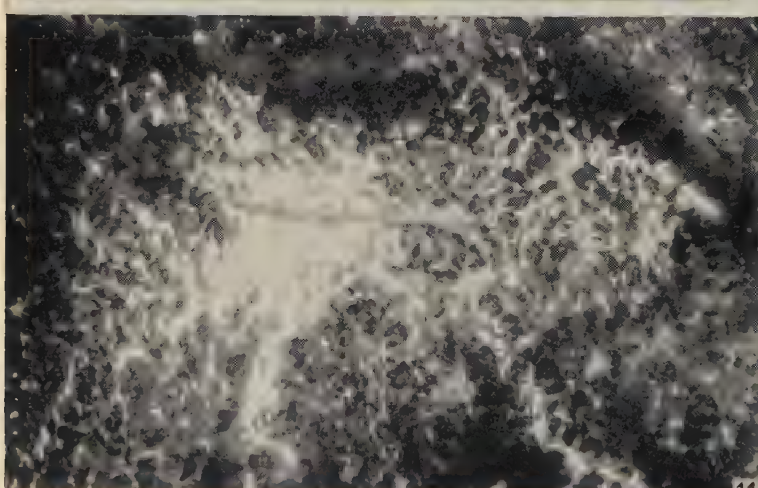
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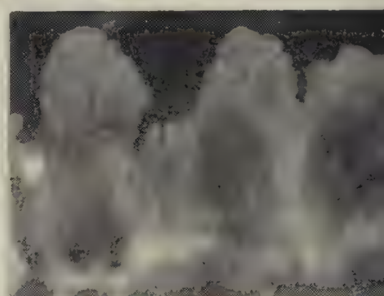
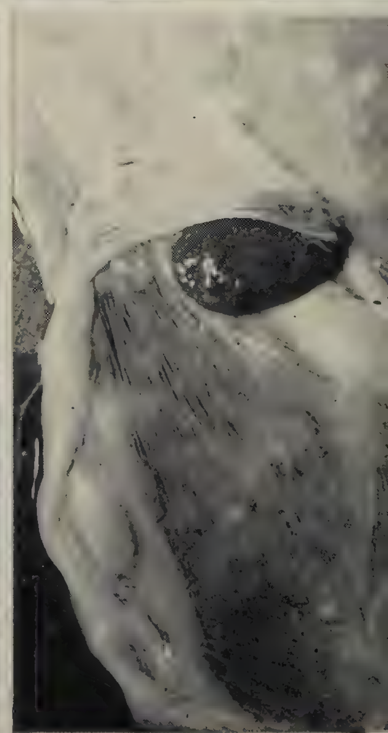
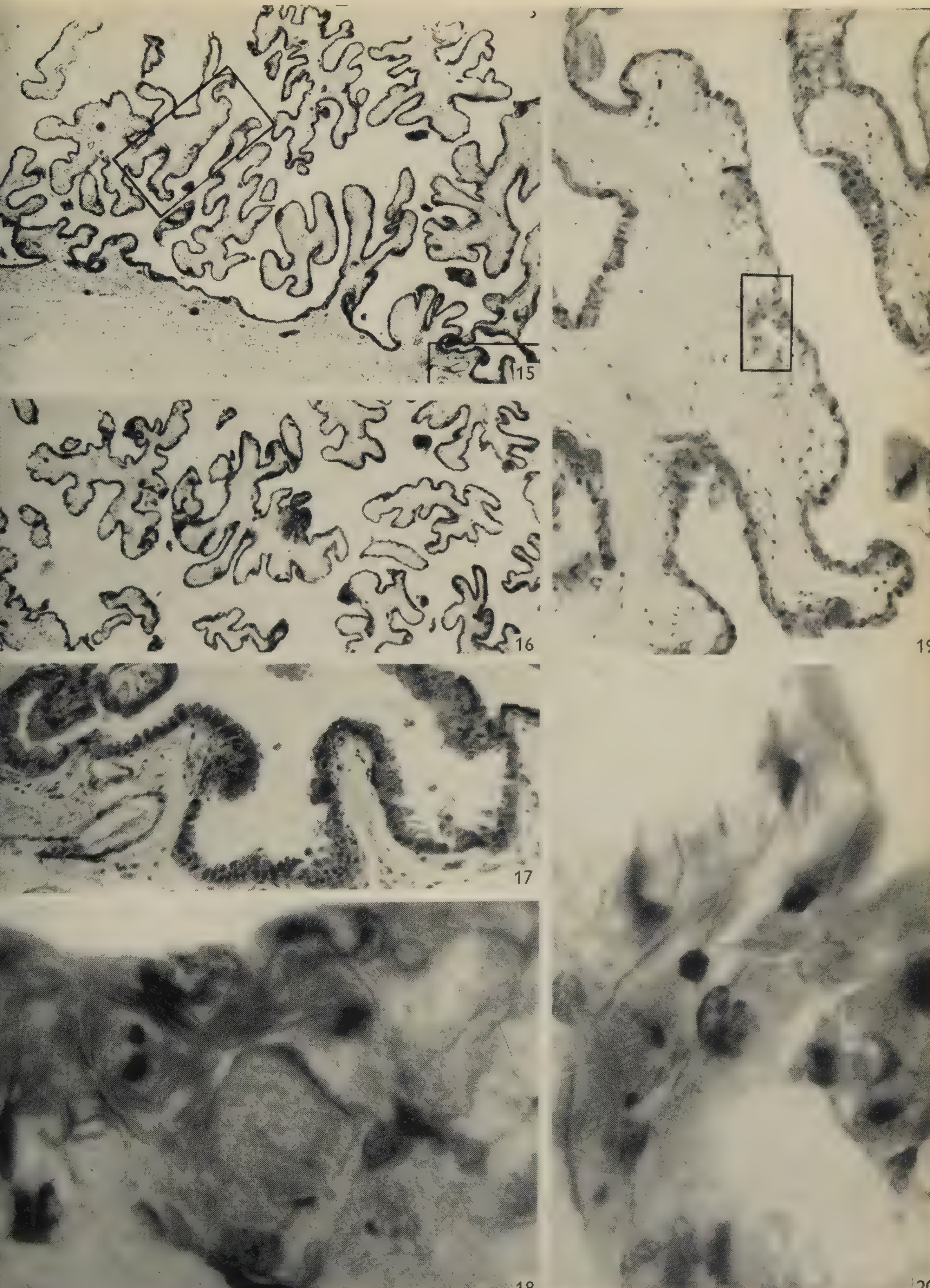




MORTON—Observations on the full-term foetal membranes of Camelidae



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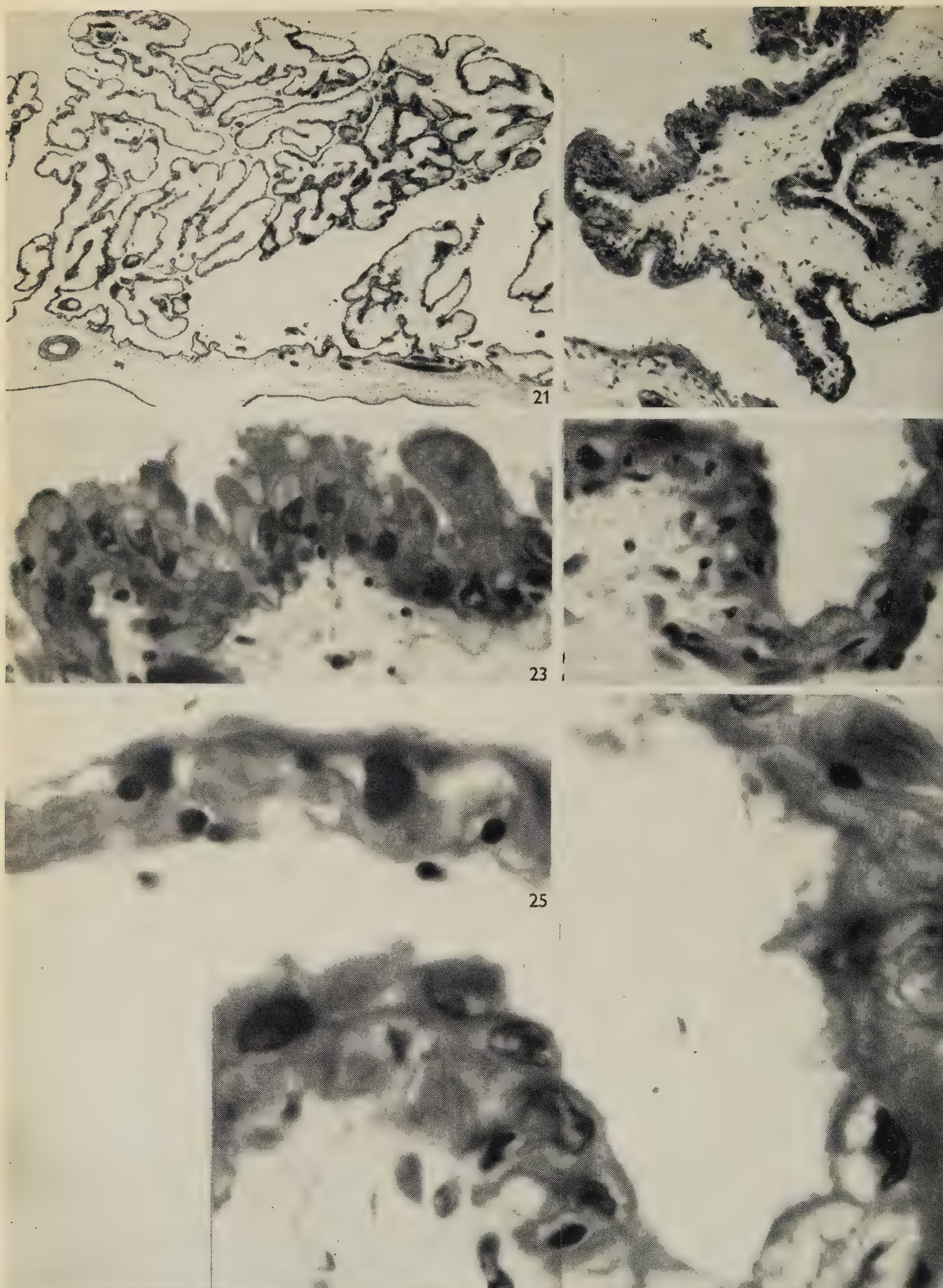

MORTON—Observations on the full-term foetal membranes of Camelidae

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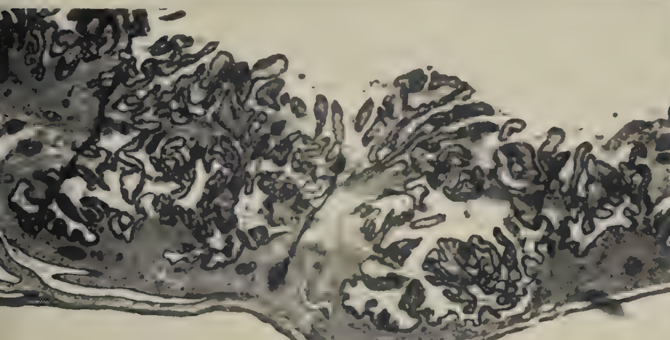

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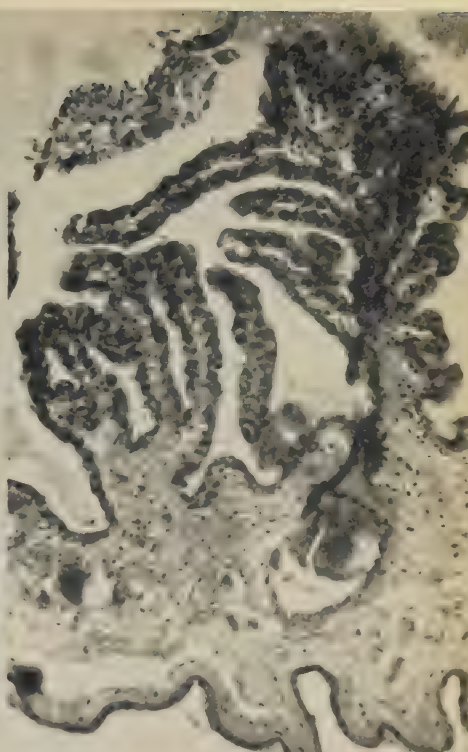

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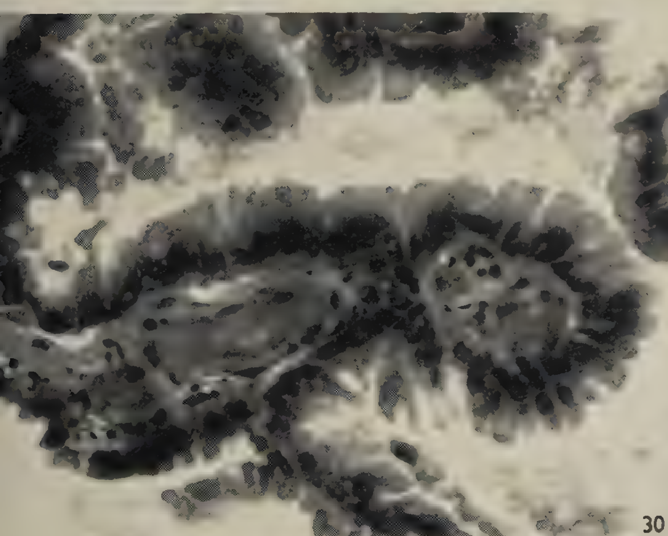

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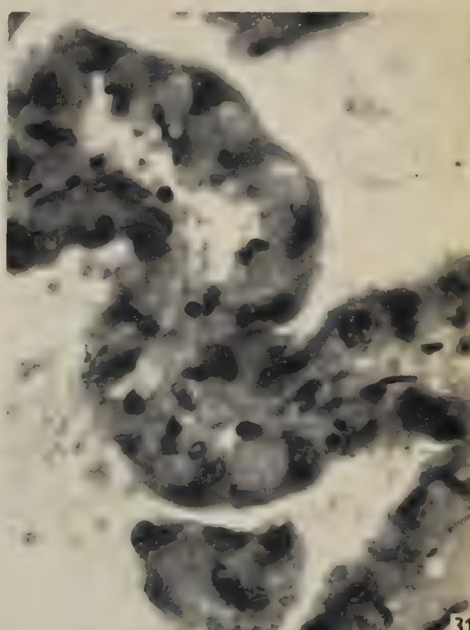

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MORTON—OBSERVATIONS ON THE FULL-TERM FOETAL MEMBRANES OF CAMELIDAE

PLATE 4

Photomicrographs of the full-term foetal placenta of the Bactrian camel (*C. bactrianus* L.).

Fig. 21. Low-power view of a T.S. of the allanto-chorion of the Bactrian camel. The bushy plicated villous tuft is attached on the left by a narrow stem to the chorionic plate. Numerous blood vessels are present in the mesenchyme between the basal trophoblast and the dark line of the allantoic lining below. ×29.

Fig. 22. Part of a villous tuft from the Bactrian placenta showing tall columnar celled cytotrophoblast (left), and masses of syncytio-trophoblast (right). The chorionic plate is below and to the left. ×132.

Fig. 23. Medium-power view of the columnar cytotrophoblast seen in fig. 22. The nuclei of the trophoblast are basally situated, and the more superficial cytoplasm is vacuolated. Intra-epithelial capillaries do not occur in such areas of cytotrophoblast. ×624.

Fig. 24. Medium-power view of the Bactrian chorion showing many intra-epithelial capillaries in the low trophoblast layer. ×624.

Fig. 25. High-power view of an area of the Bactrian chorion showing large capillaries lying within the thickness of the trophoblast. ×1400.

Fig. 26. High-power view of the same area as seen in fig. 24. The blood filled foetal capillaries on the left are deep to the trophoblastic nuclei, those on the right are more superficially placed and those at the lowest part of the figure are next to the surface of the trophoblast. ×1400.

PLATE 5

Photomicrographs of the full-term foetal placenta of the llama (*Lama glama* L.).

Fig. 27. Low-power view of a T.S. from the edge of the bare area of the chorion (see fig. 32 below and Pl. 1, fig. 6). The villous tufts have the same plicated appearance as those of the Arabian and Bactrian camels (compare with Pl. 3, fig. 15 and Pl. 4, fig. 21). The allantoic epithelial lining is below. ×29.

Fig. 28. Low-power view of a T.S. from an area of large densely massed villous tufts from the same placenta. An albuminous coagulum surrounds the free surfaces of the chorion and it was in such a coagulum that a positive phosphatase reaction was obtained. Compare with fig. 27 above. ×29.

Fig. 29. Medium power view of a large tuft from the llama chorion. The trophoblast covering the folds of the tuft is low and contains many intra-epithelial capillaries. The allantois, which is markedly folded, is lined with a flattened epithelium. ×133.

Fig. 30. High power view of avascular tall columnar cytotrophoblast of the llama chorion. A syncytial mass can be seen above. ×624.

Fig. 31. High-power view of intra-epithelial capillaries in the low trophoblast covering an adjacent villous fold. A syncytial mass is present at the bottom of the figure. ×624.

Fig. 32. Low-power view of a section across the long axis of the bare area of the llama placenta (see Pl. 1, fig. 6 and 27 above). Villous tufts are absent in the centre, and the main allantoic vessels are on the right. ×10.

THE EFFECT OF REARING INFANT RATS AT THREE ENVIRONMENTAL TEMPERATURES ON THE STRUCTURE OF SOME OF THEIR ORGANS

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INTRODUCTION

It is well known that prolonged exposure to high or low environmental temperatures results in many biochemical, morphological and endocrine changes (Sellers, 1957). There are also indications that the changes evoked by such temperatures depend to a certain extent on the age of the animal. Thus (Weiss (1957) has shown that the higher oxygen consumption of some organs in rats exposed to cold is seen only in younger animals. According to other reports exposure of young animals to cold results in shorter tail length, while exposure to high temperatures increases tail length and ear area (Michie & McLaren 1956; Čapek, Hahn, Křeček & Martínek, 1956). Histologically only the thyroid and adrenal glands have been examined, while changes in other organs have not been described in any detail. In weanling mice these two endocrine glands have been studied by Barnett (1958).

It was shown in previous papers (Čapek *et al.* 1956; Křeček *et al.* 1958) that the development of thermoregulatory mechanisms can be slowed down by raising rats at 34° C. and that a low environmental temperature also shows an early effect, e.g. on tail length, by the 10th day. Since Křeček (personal communication) found that raising rats at 33° C. results in greatly decreased skin thickness, it was thought pertinent to examine the effect of prolonged exposure to a high and low environmental temperature on the histological picture of different organs of infant rats, and to compare the changes observed with those seen in adult animals. A preliminary report of this paper was published by Vacek, Hahn & Koldovský (1958).

MATERIALS AND METHODS

Wistar rats (strain B-Konárovice) were used throughout. They were fed a stock laboratory diet and water *ad lib.* Up to the 16th-18th day of post-natal life they were kept with a mother rat. Thereafter the mother was removed. Litters were always reduced to six animals. Alternate period of low environmental temperature (3° ± 1° C.) and room temperature of 1 hr. each were used up to the 12th post-natal day, as described by Hahn (1956) Křeček & Martínek (1958). After that day animals were maintained permanently at the low temperature. The higher temperature used was 33 ± 1° C. and the room temperature was 22 ± 1°C. In all cases animals were exposed to these temperatures together with the mother on the first day after birth and remained there (with the exception of the cold room as described above) until they

were sacrificed. One group of adult rats was exposed to the three environmental temperatures for 30 days and then sacrificed. None of the animals died during the experiment. The infant animals were killed by a blow on the head when aged 10, 14, 21 and 28 days. These days were chosen because thermoregulation begins to develop on the 10th, is considerably improved on the 18th and complete on the 28th–30th day after birth (Hahn, Křeček & Křečková, 1956). Several litters from each age group were used to determine the weight of the liver, adrenals, heart and kidneys; others were used for histological studies, organs being fixed in 10% formaldehyde or in Bouin's fluid (Gray, 1954), and in cooled acetone (for examination of enzymic activity). The skin of the back and tail, the liver, the thyroid and adrenal glands were examined in paraffin sections stained with haematoxylin-eosin. The material fixed in formol was examined for fat in frozen sections treated with Oil Red O or Sudan Black in an ethyleneglycol solution. Organs were examined for alkaline phosphatase and non-specific esterase using the azocoupling method. Fixation lasted for 8 hr., embedding in paraffin 2 hr. and the inbucation period in a standard incubation medium for 15 min. α- and β-naphthol phosphate and α- and β-naphthol acetate served as substrates and Fast Red TR Fast Blue BB were used as diazotates. One adrenal gland was always examined for fat, the other embedded in paraffin and serial sections were cut for staining with haematoxylin-eosin. In all sections the width of the cortex and of the individual layers was determined. After fixing the thyroid in Bouin's fluid it was embedded in paraffin, serial sections were again made and after staining with haematoxylin-eosin the size of the follicles and the height of the epithelial cells were determined. The skin was examined for fat and enzymes, and from another sample (15×5 mm.) fixed in formol and paraffin, serial sections were made. The thickness of the stratum germinativum of the epidermis was measured in all sections stained with haematoxylin-eosin. (Only this part of the skin can be measured exactly since the stratum corneum is sometimes torn off during cutting of sections. In addition the effect of the environmental temperature is first seen in the stratum germinativum (Vacek *et al.* 1958), from which new cells are being formed.)

RESULTS

Organ weights. It is evident from Table 1 that in 18-day-old animals cold exposure has already resulted in increased relative weight of the kidneys, liver, heart and adrenals. Exposure to 33° C., on the other hand, does not change the relative weight of these organs as compared with animals raised at room temperature. In 14-day-old animals no differences are found in adrenal weights whereas the heart and kidneys are again heavier in the cold. Relative liver weight is the same for animals raised in the cold and at room temperature, and in both cases higher than for animals raised at the high temperature. At high temperature relative heart weight is also least.

Skin. In general the epidermis from the skin of the back is much thinner than that from the tail. The histological structure of the epidermis from the back in 10-day-old animals is the same for all three temperatures and the same holds good for the width of the stratum germinativum, which is $23{\cdot}3\,\mu$.

The skin of the tail, on the other hand, is much more sensitive to temperature.

In 10-day-old rats the stratum germinativum of the epidermis measures 48 μ on the average in animals kept at room temperature, 58 μ in rats kept in the cold and 45·5 μ in those kept at a high temperature.

By the 14th day after birth the stratum germinativum has decreased in thickness while the stratum corneum has increased. For the skin of the back, the average thickness of the stratum germinativum in 19 μ both for rats raised at room temperature and at 33° C. In rats raised in the cold it is somewhat greater, 21·2 μ. Differences are more evident in the skin of the tail. The stratum germinativum here measures 47·8 μ (room temperature), 45·2 μ (warm room) and 56·9 μ (cold room).

Table 1. *Organ weights (expressed as percentage of body weight) of rats adapted to* 3, 22 *and* 33° C.

Each horizontal row represents one or more experiments with animals from three litters born on the same day and divided equally into 3 groups on the first day after birth in such a way that each mother had two rats of her own and four rats from the two other mothers. Data were also obtained from animals not included in the histological examination. Figures in parentheses denote number of animals in each group. * = $P < 0{\cdot}01$, ** = $P < 0{\cdot}05$—statistically significant against animals raised at 23° C.

Age (days)	Kidney			Adrenals		
	3° C.	23° C.	33° C.	3° C.	23° C.	33° C.
14	1·25* ± 0·022 (8)	1·16 ± 0·020 (8)	1·16 ± 0·025 (6)	0·027 ± 0·003	0·032 ± 0·002	0·030 ± 0·003
14	1·37* ± 0·045 (5)	1·08 ± 0·350 (8)	1·20 ± 0·045 (6)	—	—	—
14	1·17 ± 0·022 (6)	—	1·11 ± 0·030 (8)	0·038 ± 0·004	—	0·037 ± 0·003
18	1·20 ± 0·066 (6)	1·01 ± 0·06 (8)	1·02 ± 0·051 (6)	0·046** ± 0·0038	0·032 ± 0·0028	0·037 ± 0·0025
19	1·32* ± 0·048 (4)	1·10 ± 0·046 (8)	—	0·045** ± 0·0020	0·036 ± 0·003	—
20	1·32* ± 0·050 (7)	1·10 ± 0·040 (8)	1·18 ± 0·026 (8)	0·044** ± 0·0023	0·0375 ± 0·0016	0·0335 ± 0·003

	Liver			Heart		
	3° C.	23° C.	33° C.	3° C.	23°C.	33° C.
14	3·66 ± 0·118	3·29 ± 0·136	3·32 ± 0·133	0·547 ± 0·025	0·56 ± 0·002	0·476* ± 0·012
14	3·25 ± 0·105	3·44 ± 0·15	2·87 ± 0·4	0·661* ± 0·031	0·48 ± 0·021	0·438 ± 0·024
14	3·10 ± 0·13	—	2·80 ± 0·128	0·47 ± 0·024	—	0·38 ± 0·018
18	4·310 ± 0·23	3·45 ± 0·21	4·05 ± 0·28	0·82* ± 0·30	0·62 ± 0·024	0·44* ± 0·026
19	5·50* ± 0·26	4·4 ± 0·20	—	0·585* ± 0·021	0·480 ± 0·023	—
20	5·4* ± 0·20	4·3 ± 0·19	4·16 ± 0·22	0·63* ± 0·037	0·45 ± 0·018	0·49 ± 0·017

These differences remain about the same on the 21st day when the thickness of the stratum germinativum of the skin of the back is 18·5 μ (room temp.), 17·9 μ (warm room) and 20·3 μ (cold room). For the tail the figures are 47·6 μ (room temp.), 45·4 μ (warm room) and 56·5 μ (cold room). Conditions are similar on the 28th day but the stratum germinativum from rats in the warm room (skin of back) is thinner still (see Table 2).

A different picture is seen in adult animals exposed to high or low temperature for one month. No differences between the three groups are seen in the skin of the back whereas the stratum germinativum of the tail has a greater thickness in the animals exposed to cold (Table 2).

Great differences have been found in the amount of fatty tissue in the dermis. In infant rats raised at room temperature (10, 14, 21 and 28 days) the dermis of the skin of the back regularly contains small lobules of fat cells and also single fat cells contained in the spaces between hair follicles (Pl. 1, figs. 1–9). The skin from rats raised at 33° C. contains the largest number of fat cells. In addition to the large number of these cells found in the interfollicular spaces in the dermis large lobules of fat are seen in the subcutaneous tissue; these are absent in the skin of rats raised at room temperature. In rats raised in the cold, on the other hand, the amount of fat is considerably reduced. The dermis contains much less fat than in rats raised at room temperature and only occasional fat cells are found in the interfollicular spaces.

Table 2. *Thickness of the stratum germinativum of the skin of the back and of the tail in animals adapted to* 3, 22 *and* 33° C.

Age (days)	Skin of the back			Skin of the tail		
	22° C.	33° C.	3° C.	22° C.	33° C.	3° C.
10	23·3 ± 0·31	23·4 ± 0·32	23·2 ± 0·33	48·0 ± 0·65	45·4 ± 0·53	58·0 ± 0·85
14	19·1 ± 0·23	18·9 ± 0·25	21·2 ± 0·32	47·8 ± 0·73	45·2 ± 0·67	56·9 ± 0·61
21	18·5 ± 0·14	17·9 ± 0·27	20·3 ± 0·30	47·6 ± 0·64	45·4 ± 0·41	56·5 ± 0·67
28	18·3 ± 0·25	16·6 ± 0·24	19·9 ± 0·24	47·0 ± 0·61	45·3 ± 0·63	55·3 ± 0·44
Adult	20·8 ± 0·34	20·6 ± 0·36	21·0 ± 0·31	45·6 ± 0·68	45·2 ± 0·51	50·5 ± 0·89

The skin of the tail of young rats (10, 14, 21 and 28 days of age) has fewer fat cells. Occasional fat cells or groups of cells are found in the interfollicular spaces. There is no difference between rats raised at 22 and 33° C. but those raised at 3° C. contain fewer fat cells. In adult animals fat cells are practically absent from the interfollicular spaces but large lobes of fat cells are found in the subcutaneous tissue. These form a nearly continuous layer of fatty tissue 220 μ thick in rats kept at room temperature and in the warm room, and 470 μ thick in rats kept in the cold for 1 month. Thus here the picture is exactly the opposite to that found in young rats.

In infant rats aged 10, 14 21 and 28 days a positive reaction for non-specific esterase is found in the fat cells, the external epithelial sheath of hair follicles and the sebaceous glands. The effect of temperature is seen in the sebaceous glands, those from rats kept at a high temperature showing a more pronounced reaction than the other two groups. The same results have been obtained in adult animals and again activity in the sebaceous glands from rats kept in the warm environment is highest.

Liver. The amount of fat and its localization in the liver of 10-day-old animals depends on the environmental temperature. The liver from rats kept at room temperature contains a relatively large amount of fat which is present in the cells in the form of small droplets. The peripheral zone of liver lobules contains the largest amount of fat (Pl. 2, fig. 16). The liver of rats kept in the cold contains much less fat; in fact this substance is practically absent and only occasionally do we find groups of cells containing small fat droplets in their cytoplasm (Pl. 2 fig. 18). The amount of fat is the same in the livers of rats kept at 22 and 33° C., but in the latter case most of the fat is found in the central zone of the lobules, while the peripheral

part and the part surrounding the central veins has little fat (Pl. 2, fig. 17). The same pictures are seen in rats aged 14, 21 and 28 days.

In adult rats there is less fat in the liver and most of it is found in the peripheral part of the lobules. There is no difference either in the localization or the amount of fat between the three groups (Pl. 3, figs. 19, 20). Only the bile ducts show a positive reaction for alkaline phosphatase. There are no differences in this respect between infant and adult rats or between the three experimental groups. The cytoplasm of liver cells shows a strong reaction for non-specific esterase, activity being highest in the part surrounding the central vein, and this part is considerably more extensive in rats kept in the cold than in the other two groups (Pl. 2, figs. 10–15). In adult animals the enzyme is localized in a similar way but cold has no effect.

Thyroid gland. The thyroid of 10-day-old animals reared at 22 or 3° C. has follicles of very variable size. In addition to follicles, the lumen of which contains colloid material, many solid islets, without a lumen, are found and these are the basis of additional follicles. They are especially visible in rats raised in the cold (Fig. 28). Table 3 shows the average follicle size and the height of their epithelium. This is less for animals reared at room temperature than for those raised in the cold. This undoubtedly indicates increased thyroid activity. In these animals numerous mitoses of epithelial cells are also found. In animals raised in the warm room there are very few solid epithelial islets but many more large follicles and the epithelial layer is much smaller in height (Pl. 4, fig. 29). This indicates decreased thyroid activity. These differences are more apparent in 14-day-old animals (Table 3). Differences between animals from the cold and room temperature groups are less than those between animals reared at room temperature and in the warm room. Again, many mitotic divisions are observed in the animals from the cold room and also more solid epithelial islets are found.

In 21- and 28-day-old animals the average follicle size increases but differences between the three groups remain the same (Table 3).

Table 3. *Average follicle size and height of epithelium of the thyroid glands of rats adapted to three environmental temperatures*

Age (days)	Follicle size (μ)			Epithelial height (μ)		
	22° C.	33° C.	3° C.	22° C.	33° C.	3° C.
10	23·2 ± 0·32	31·6 ± 0·45	23·2 ± 0·25	5·8 ± 0·12	3·5 ± 0·03	7·4 ± 0·14
14	25·2 ± 0·41	35·3 ± 0·53	24·1 ± 0·36	6·2 ± 0·83	3·5 ± 0·31	7·5 ± 0·74
21	43·6 ± 0·79	49·3 ± 0·87	42·6 ± 0·74	7·1 ± 0·82	3·7 ± 0·06	7·7 ± 0·74
28	44·8 ± 0·74	50·2 ± 0·75	43·1 ± 0·72	7·6 ± 0·03	4·2 ± 0·05	8·3 ± 0·12
Adult	55·7 ± 0·93	61·8 ± 0·87	54·1 ± 0·81	8·8 ± 0·14	5·3 ± 0·79	9·6 ± 1·54

In adult animals there is only a small difference between animals kept in the cold and at room temperature. The follicle size of animals kept at 33° C. is more uniform and larger and the epithelium is lower (Pl. 4, fig. 32).

Adrenal gland. In 10-day-old animals a sexual difference is already seen. The average width of the cortex is somewhat smaller in males than in females (Table 4) but the zona glomerulosa is wider in the male. It is strongly sudanophilic. In the

male rat the zona glomerulosa is separated from the zona fasciculata by a sudanophobe zone of 25μ average width. This is not present in female animals. No differences in the width of the cortex and the individual zones have been found between the three experimental groups. The superficial part of the zona fasciculata is rich in lipoid material but contains less than the zona glomerulosa. In animals raised at room temperature and in the cold the fat content decreases, beginning with the intermediate third of the zona fasciculata while the innermost third is practically free of fat. The zona reticularis is narrow and indistinct, and also contains little lipoid material. In rats raised at 33° C., on the other hand, the zona fasciculata contains much more fat. It is densely distributed in the superficial and intermediate thirds and only begins to disappear in the innermost third (Pl. 3, figs. 21–23).

Table 4. *Width of adrenal cortex, glomerular zone and transitional zone in male and female rats adapted to three environmental temperatures* (μ)

Age (days)	Width of cortex male			Width of cortex female		
	22° C.	33° C.	3° C.	22° C.	33° C.	3° C.
10	330 ± 4	324 ± 5	328 ± 3	350 ± 5	347 ± 4	353 ± 4
14	450 ± 7	453 ± 8	448 ± 6	510 ± 9	514 ± 10	512 ± 8
21	550 ± 9	548 ± 9	553 ± 4	600 ± 7	604 ± 11	607 ± 4
28	620 ± 11	545 ± 8	560 ± 7	670 ± 10	550 ± 10	572 ± 4
Adult	950 ± 13	943 ± 15	961 ± 8	990 ± 6	1020 ± 16	1013 ± 14

Age (days)	Width of transitional zone*	Width of glomerular zone	
		Male	Female
10	25 ± 0·3	50·0 ± 0·9	45·0 ± 1·0
14	30 ± 0·4	30·0 ± 0·5	20·0 ± 0·3
21	20 ± 0·5	61·0 ± 1·0	51·0 ± 1·0
28	—	62·0 ± 1·0	49·0 ± 0·4
Adult	30 ± 0·3	69·0 ± 0·8	68·0 ± 0·5

* Only present in male animals.

Similar conditions are found in the adrenals of 14-day-old animals. The cortex is again wider in the female, the zona glomerulosa wider in the male and sudanophilic in both sexes. A sudanophobe zone is found in the males (Table 4). In rats reared in the warm room the zona fasciculata again contains more fat.

In 21-day-old animals the zona glomerulosa has increased in width (Table 4). The sudanophilic zona glomerulosa is separated from the zona fasciculata by a narrow sudanophobe zone in both sexes and again fat is most abundant in the zona fasciculata of rats reared at 33° C. In 28-day-old animals conditions are similar but in contrast to younger rats the effect of temperature is also seen in the width of cortex (Table 4). The latter is narrower in animals raised in the cold or warm room than in rats reared at room temperature. Again the zona fasciculata of rats from the warm room contains the largest amount of fat.

In adult animals kept in the cold the zona fasciculata contains more fat than in animals kept at 22 or 33° C. In other words, the reaction is exactly the opposite to that in infant animals (Pl. 3, figs. 21–26).

DISCUSSION

It is evident from the above results that exposure to cold has a variable effect on rats, depending on their age. Relative organ weights indicate that during the period when thermoregulation is not yet fully developed even room temperature causes an increase in the relative weight of the heart and liver since the temperature in the nest is much higher (Čapek *et al.* 1956). These results may be compared with those of Heroux & Gridgeman (1958) for adult rats.

Histological analysis of the skin shows that the skin of the tail is very sensitive to changes in temperature even in 10-day-old animals. The skin of the back, on the other hand, reacts somewhat later in life, differences due to cold exposure appearing sooner than those caused by the high temperature. In adult animals only the skin of the tail reacts to cold exposure. This is in agreement with the work of Davis & Mayer (1955) who have shown that the tail is the most receptive organ for temperature changes. Changes in the fat content of the skin suggest that whereas in infant animals, whose physical thermoregulatory mechanisms are not fully developed (Hahn *et al.* 1956), no fat is laid down in the skin on exposure to cold, exactly the opposite occurs in adult animals. It thus appears that the role of fat in thermoregulation in young animals is mainly to serve as fuel, while in adult ones it also plays an insulating role. Since infant rats exposed to 33° C. need no energy for thermoregulation the large amount of fat contained in milk finds its way into the skin and stays there. It is, of course, also possible that lipogenesis is increased at the high temperature, a supposition that is perhaps indicated by the higher esterase activity in the skin of animals raised in the warm room.

It is difficult to correlate the above changes with those found in the liver. Lipogenesis must be much higher in adult animals since their food contains much less fat than mother's milk. Perhaps it occurs mostly in adipose tissue itself, as indicated by the work of Hausberger & Milstein (1955), so that no changes are seen in the liver. This must mean of course that adult rats exposed to cold are very well adapted, since shorter exposure to cold inhibits lipogenesis (Masoro & Felts, 1959). Changes in the distribution of fat and esterase activity in the liver found in infant rats might well be due to changes in the transport of fat but further work is required to elucidate this point.

Changes observed in the thyroid gland indicate decreased activity at the high temperature. Surprisingly enough, differences between animals living at room temperature and in the cold are slight, although it is well known that thyroid activity is increased in the cold (Ring, 1936). It is also evident that the thyroid continues to develop for some time after birth and that changes due to the two extreme temperatures are more pronounced in infant rats than in adult animals. Barnett (1958) also has found similar changes in infant mice exposed to cold.

The histological appearance of the adrenals, although difficult to interpret, suggests that the adrenals play a role in the adaptation of rats to low, and perhaps also to high, temperatures. Some authors report increased size and weight of adrenals after cold exposure (Reineck, 1928; Selye, 1936). Changes in lipid content of the cortex are also reported (Hoskins & Bernstein, 1939). It appears that short-term exposure results in a transient decrease in lipid content (Robinson & Yoffey, 1950; Dosne & Dalton, 1941; Flexner & Grollman, 1939), while after long-term

exposure lipid content increases (Selye, 1936). In the present experiment more fat has been found in the adrenals of adult animals exposed to cold and this is in agreement with the literature; in infant rats, however, most lipid material has been found in the adrenals of warm-adapted animals. A tentative explanation for this fact might be that infant rats require more effort to adapt to a low temperature and that this is reflected in the adrenals. It is also interesting to note that sexual differences are already apparent in 10-day-old animals.

Our experiments demonstrate that adaptation proceeds differently in infant and adult rats, evidently being dependent on the metabolic and regulatory mechanisms available to the animal. It appears that infant animals must adapt themselves to varied environmental temperatures, including room temperature, and that the adaptation observed in adult animals differs from that in infant animals by the fact that it occurs in animals already adapted to one particular environmental temperature. Infant animals adapt themselves for the first time. It would be interesting to know if there are any differences between adult rats reared in heat or cold all their life and animals exposed to heat or cold later in life. Unfortunately it has not been possible to carry out such an experiment.

It is realized that the mother forms an integral part of the environment for infant animals. At this stage it cannot be decided to what extent adaptation of the mother (in regard to milk production, for example) is responsible for the changes observed in the infant, after both have been exposed to unusually low or high temperatures.

SUMMARY

Rats were raised from birth at three environmental temperatures (3, 22 and 33° C.) for 10, 14, 21 and 28 days and compared with adult animals kept at those environmental temperatures for 30 days. The thyroid and adrenal glands, the skin of the back and tail, and the liver were examined histologically and histochemically. Organ weights were also determined.

1. The stratum germinativum of the skin of the tail is always thicker in animals raised in the cold than in the other two groups. In animals raised at 33° C. it is thinner than in those raised at 22° C. but this difference is much smaller and not apparent in adult animals.

2. Changes are similar in the skin of the back but only appear in animals aged 14 days and older ones and the difference between the groups is much smaller. In young animals there is hardly any fat in the skin in animals raised at 3° C. and much fat in those raised at 33° C. Exactly the opposite is found in adult animals.

3. The fat content of the livers of young rats depends on the environmental temperature, being very low at 3° C. and higher at the two higher temperatures. In adult animals no such differences are seen. Esterase activity is highest in young animals kept in the cold but again this is not so for adult rats.

4. The thyroid gland continues to develop after birth for some time and this development depends on the environmental temperature. The effect of heat and cold is most apparent in the youngest animals and least visible in adult ones.

5. In 10-day-old animals a sexual difference in the width of the adrenal cortex is already present. These differences are described in detail. In young animals the

zona fasciculata contains most sudanophilic material in animals raised at 33° C., whereas in adult animals this is observed in the cold environment.

6. It is concluded that adaptation to temperature proceeds differently in infant and adult animals.

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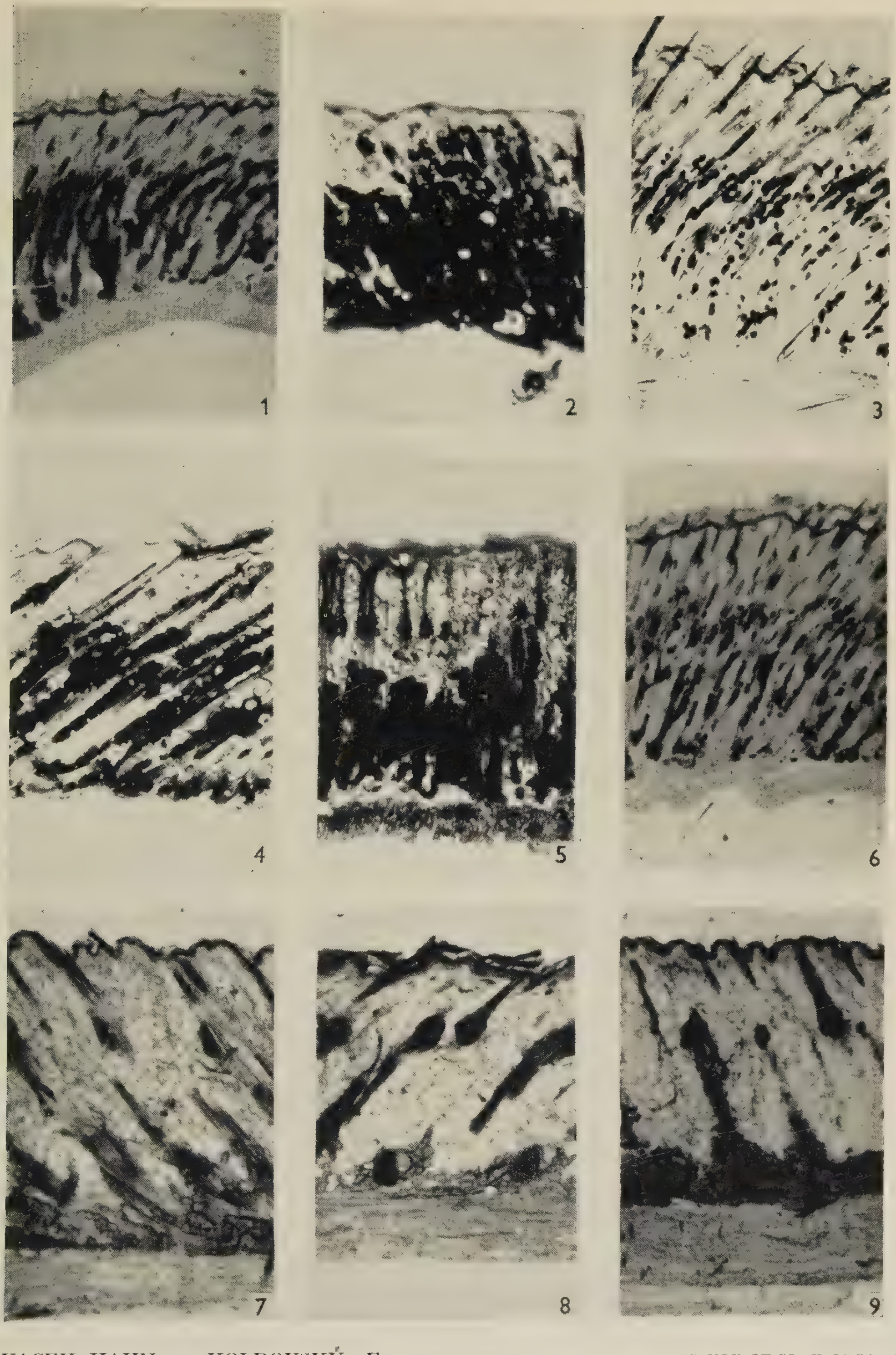

VACEK, HAHN AND KOLDOVSKÝ—EFFECT OF TEMPERATURES ON STRUCTURE OF SOME ORGANS

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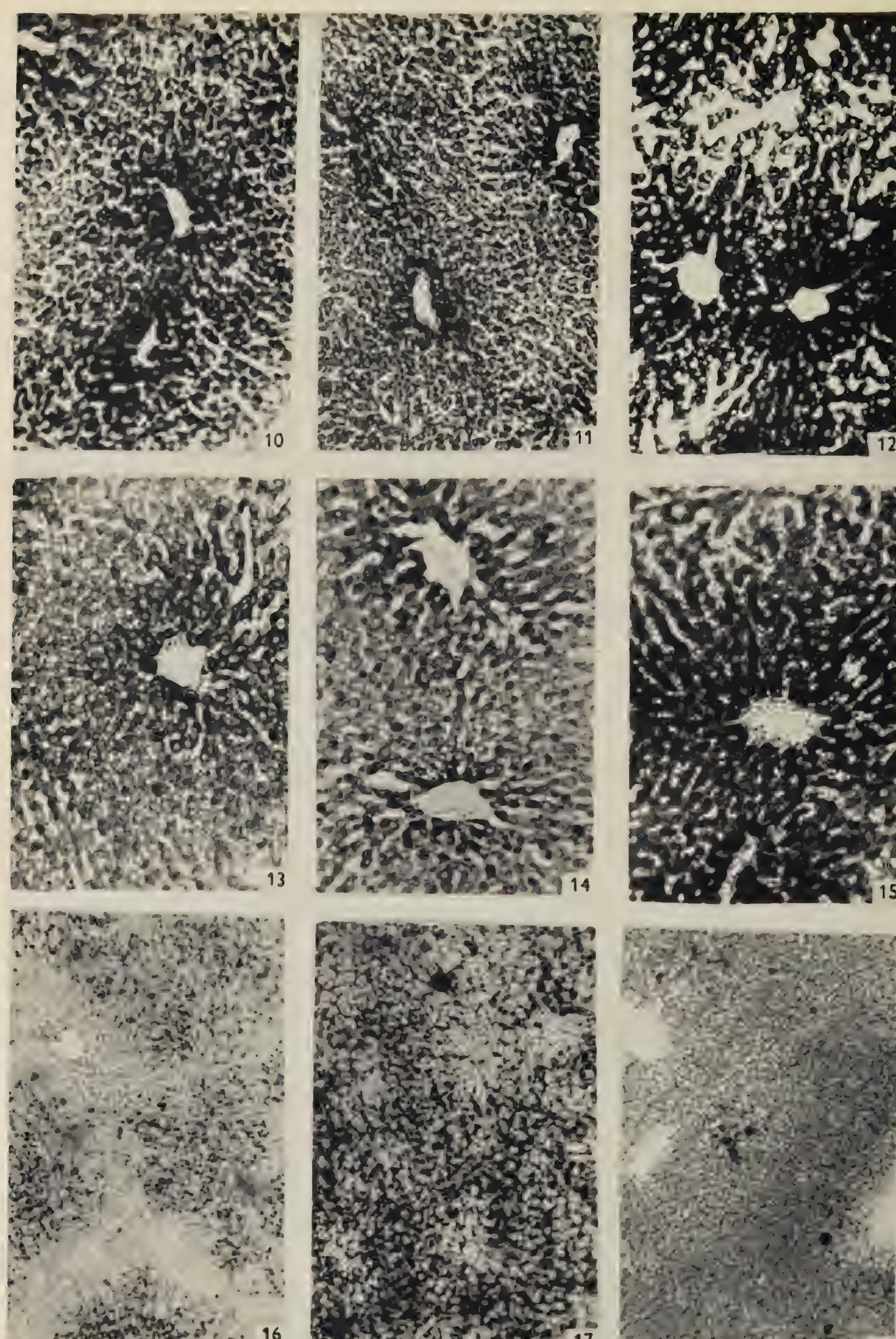

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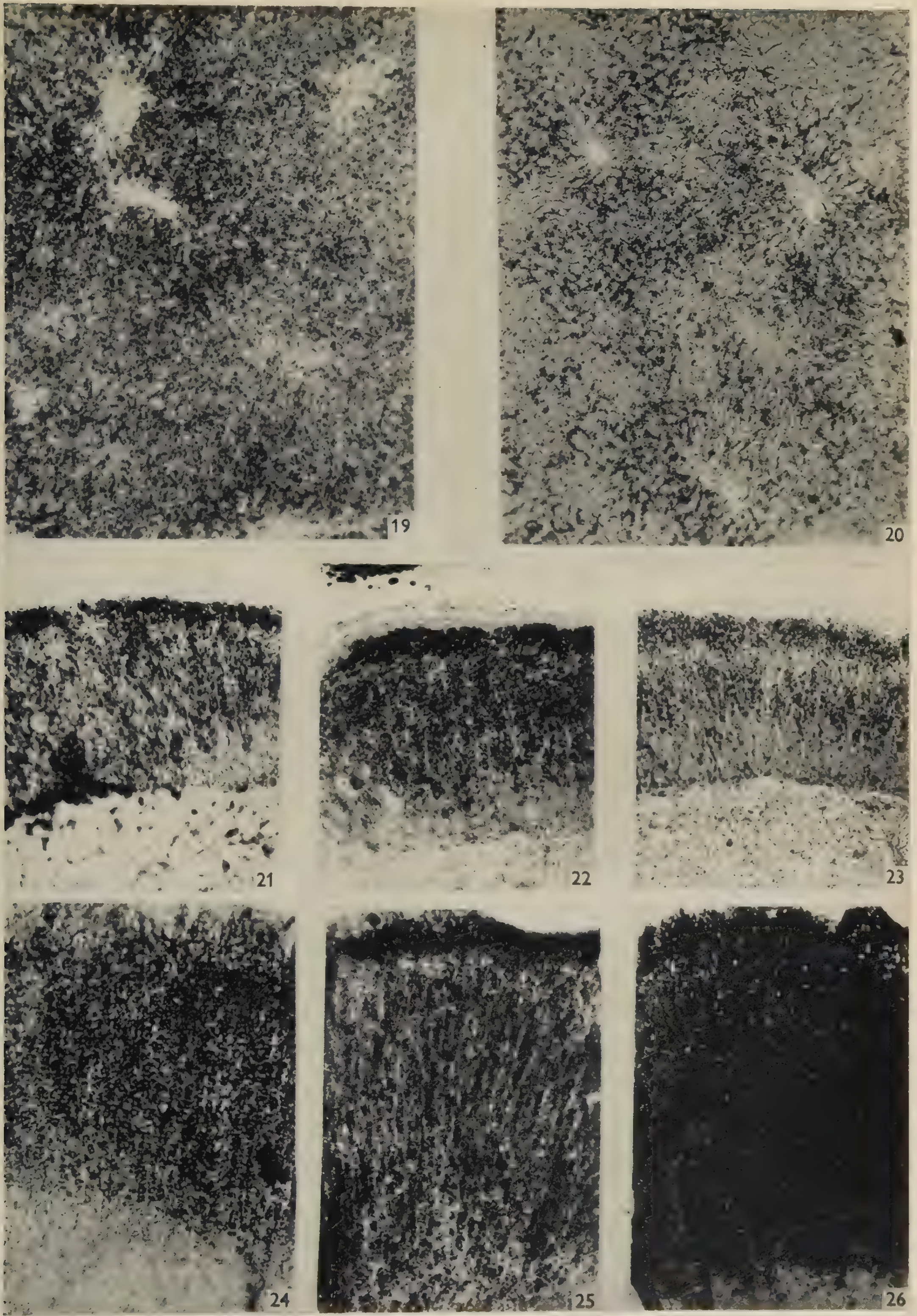

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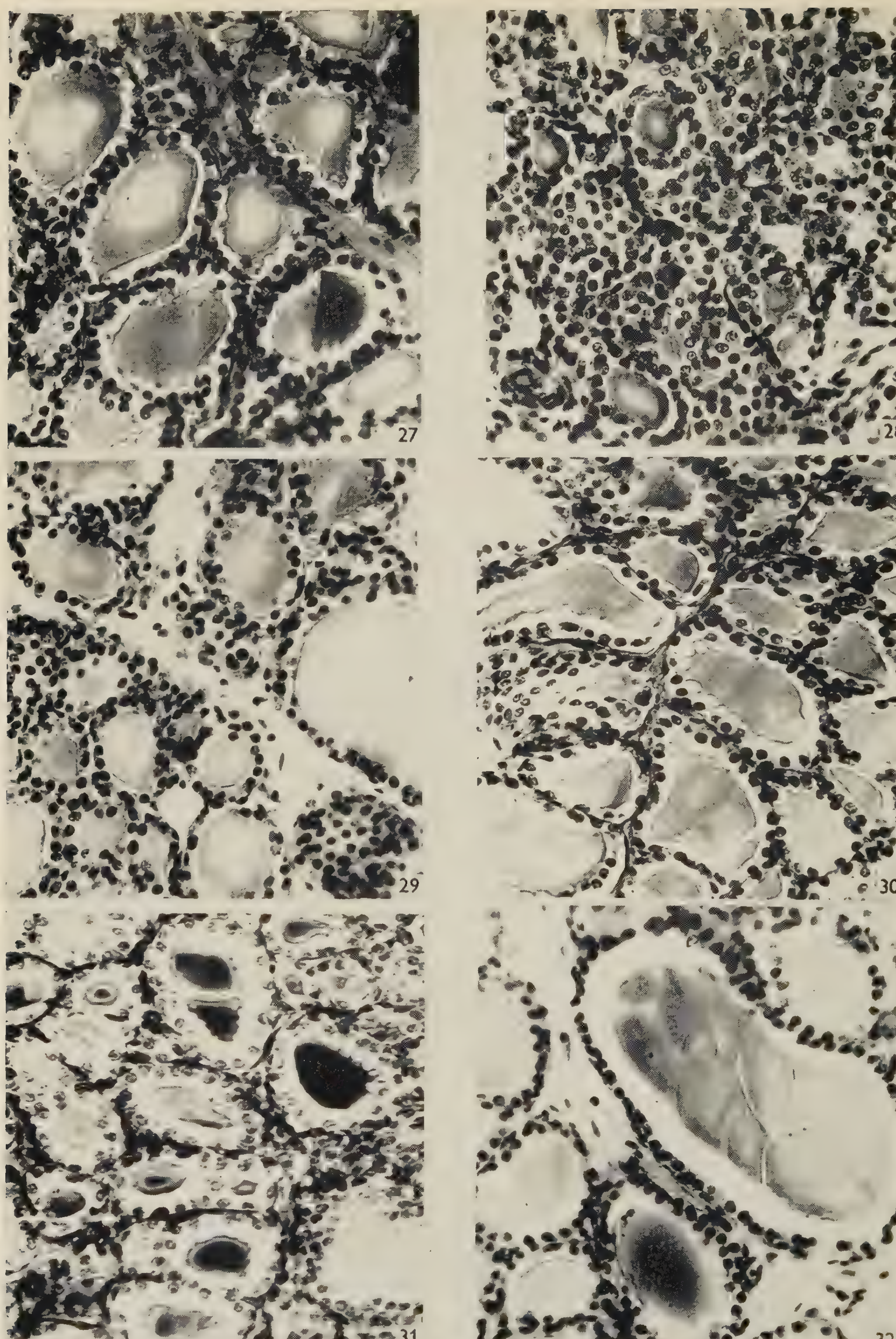

VACEK, HAHN AND KOLDOVSKÝ—EFFECT OF TEMPERATURES ON STRUCTURE OF SOME ORGANS

EXPLANATION OF PLATES

Plate 1

Fig. 1. Skin of back of 10-day-old animal raised at room temperature (22° C.). Oil Red O, ×25.
Fig. 2. Skin of back of 10-day-old animal raised at 33° C. Oil Red O, ×25.
Fig. 3. Skin of back of 10-day-old animal raised at 3° C. Oil Red O, ×25.
Fig. 4. Skin of back of 21-day-old animal raised at 22° C. Oil Red O, ×25.
Fig. 5. Skin of back of 21-day-old animal raised at 33° C. Oil Red O, ×25.
Fig. 6. Skin of back of 21-day-old animal raised at 3° C. Oil Red O, ×25.
Fig. 7. Skin of back of adult animal raised at 22° C. Oil Red O, ×25.
Fig. 8. Skin of back of adult animal raised at 33° C. for 30 days. Oil Red O, ×25.
Fig. 9. Skin of back of adult animal raised for 30 days at 3° C. Oil Red O, ×25.

Plate 2

Fig. 10. Liver of 10-day-old animal raised at 22° C. Non-specific esterase. α-naphthol acetate, Fast Red TR, ×150.
Fig. 11. Liver of 10-day-old animal raised at 33° C. Non-specific esterase. α-naphthol acetate, Fast Red TR, ×150.
Fig. 12. Liver of 10-day-old animal raised at 3° C. Non-specific esterase. α-naphthol acetate, Fast Red TR, ×150.
Fig. 13. Liver of 21-day-old animal raised at 22° C. Non-specific esterase. α-naphthol acetate, Fast Red TR, ×150.
Fig. 14. Liver of 21-day-old animal raised at 33° C. Non-specific esterase. α-naphthol acetate, Fast Red TR, ×150.
Fif. 15. Liver of 21-day-old animal raised at 3° C. Non-specific esterase. α-naphthol acetate, Fast Red TR, ×150.
Fig. 16. Liver of 21-day-old animal raised at 22° C. Oil Red O, ×110.
Fig. 17. Liver of 21-day-old animal raised at 33° C. Oil Red O, ×110.
Fig. 18. Liver of 21-day-old animal raised at 3° C. Oil Red O, ×110.

Plate 3

Fig. 19. Liver of adult animal raised at 3° C. for 30 days. Oil Red. O, ×110.
Fig. 20. Liver of adult animal raised at 33° C. for 30 days. Oil Red. O, ×110.
Fig. 21. Adrenal gland of 21-day-old animal raised at 22° C. Oil Red. O, ×55.
Fig. 22. Adrenal gland of 21-day-old animal raised at 33° C. Oil Red O, ×55.
Fig. 23. Adrenal gland of 21-day-old animal raised at 3° C. Oil Red O, ×55.
Fig. 24. Adrenal gland of adult animal raised at 22° C. Oil Red O, ×65.
Fig. 25. Adrenal gland of adult animal raised at 33° C. for 30 days. Oil Red O, ×65.
Fig. 26. Adrenal gland of adult animal raised at 3° C. for 30 days. Oil Red O, ×65.

Plate 4

Fig. 27. Thyroid of 10-day-old animal raised at 22° C. Haematoxylin-eosin, ×600.
Fig. 28. Thyroid of 10-day-old animal raised at 3° C. Haematoxylin-eosin, ×600.
Fig. 29. Thyroid of 10-day-old animal raised at 33° C. Haematoxylin-eosin, ×600.
Fig. 30. Thyroid of adult animal raised at 22° C. Haematoxylin-eosin, ×400.
Fig. 31. Thyroid of adult animal raised at 3° C. for 30 days. Haematoxylin-eosin, ×400.
Fig. 32. Thyroid of adult animal raised at 33° C. for 30 days. Haematoxylin-eosin, ×400.

MARROW VASCULARIZATION AND OESTROGEN-INDUCED ENDOSTEAL BONE FORMATION IN MICE

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Kyes & Potter (1934) were the first to demonstrate the relationship between ovarian follicular maturation and cyclical ossification in the medullary cavities of female pigeons. Since then it has been established that the administration of oestrogens can result in the formation of considerable quantities of endosteal new bone in birds generally (Zondek, 1937; Pfeiffer & Gardner, 1938; Landauer & Zondek, 1944). Amongst mammals, however, the mouse would appear to be unique in responding to oestrogens by a progressive formation of new bone trabeculae directly from medullary connective tissue elements (Gardner & Pfeiffer, 1938; Sutro, 1940; Urist, Budy & McLean, 1948). In the rat, oestrogens merely inhibit metaphyseal bone resorption (Day & Follis, 1941; Lippman & Saunders, 1943).

The fate of the marrow blood vessels in the presence of new bone formation evoked by oestrogen administration in birds and mice is uncertain. Bloom, Bloom & McLean (1941) stated that the marrow blood vessels are undisturbed in the pigeon; Riddle, Ranch & Smith (1944) investigating the same animal concluded that all large medullary blood vessels disappeared. In the mouse Urist, Budy & McLean (1950) claimed that endosteal bone formation results in a disruption of the vascular pattern and organization of the bone marrow.

In view of the general uncertainty about the morphogenetic influences of vascular patterns on bone formation and vice versa, it was decided to re-examine the phenomenon of bone formation in the medullary cavities of the mouse after oestrogen treatment, with particular reference to the fate of the blood vessels.

MATERIALS AND METHODS

The mice used in the experiment were all albinos but otherwise genetically heterogeneous. Litters were reared and weaned at 3 weeks when they were separated out into male and female cages using a split litter experimental design, so that littermate controls were available for all oestrogen-treated mice, which were examined at intervals of 2, 4, 6, 8, 10 and 12 weeks after the start of treatment.

The oestrogen employed was oestradiol monobenzoate (Ovocyclin—Ciba) supplied in 1 ml. ampoules containing 1 mg. (10,000 i.u. or 1000 μg) of the active substance. Mice were weighed daily and injected subcutaneously with oestrogen in a dosage of 40 i.u./g. body weight. Treatment was given for 5 days each week, so that taking the average weight of an oestrogen-treated mouse during the whole experimental period as approximately 15 g. and an oestrus dose for a mouse as $0{\cdot}1\mu$g. (Shorr, 1948), then each mouse received a dose of oestrogen of the order of 300 μg. per week or 3000 times an oestrus dose. Such an unphysiological dosage, not unusual for work in

this field, served to provide mouse femora and tibiae in which the marrow cavities were in an advanced state of ossification in a reasonably brief period, permitting vascular studies to be undertaken. In the course of the investigation only three mice died out of fifty-two treated with oestrogen.

At the selected intervals, some of the mice were injected *per cordem* with indian ink suspension until the skin of the paws had been blackened. The preparations were fixed whole in 2% formaldehyde in normal saline solution and radiographed in order to follow the progress of new bone formation. Decalcification followed in 5% nitric acid–2% formaldehyde solution, after which the hind limbs were embedded in paraffin and longitudinal sections cut through the femur and tibia at 10μ. These were stained with haematoxylin and eosin or with Mallory's trichrome stain. Other mice were injected intravascularly with 3 ml. Thorotrast solution *per cordem* followed by a 50% Micropaque barium sulphate suspension (Damancy and Co.) until optimal filling of the hind limbs was judged to have taken place by direct inspection of the muscles of the thigh. In this way it was intended that Micropaque, which in the dilution employed can reach a capillary bed, would push before it the Thorotrast solution which is known to be capable of passing through the smallest vascular channels (Brookes & Harrison, 1957). After fixation the whole mouse skeleton was radiographed on Kodaline film. Selected hind limb specimens were also microradiographed using a micro-focus X-ray unit and Kodak maximum resolution plates. These were then decalcified, and embedded in 20% L.V.N. (Chesterman & Leach, 1949). Sections were cut sagittally along the length of the femur and tibia at 250 and 15μ alternately. The thick sections were examined microradiographically; the thin sections were stained with haematoxylin and eosin and examined histologically. In this way, the one and the same bone provided angiographic and histological observations of the bone marrow. Indian ink and barium sulphate preparations were made from the hind limbs of control mice in the manner outlined above, which were examined histologically and microradiographically in the same way as the experimental material

RESULTS

Endosteal bone formation. Radiography of the undecalcified mouse posterior extremities showed that, after 2 weeks of oestrogen treatment, new bone, visualized as an increase in radiopacity, was formed in the bone extremities adjacent to the knee joint. Thereafter medullary bone accretion extended from each extremity of both tibia and femur and spread through the bone marrow towards the point of entry of the principal nutrient artery into the medulla, this region of the marrow being the last to show ossification in either bone. After 8 weeks of oestrogen treatment, the marrow cavity of the mouse tibia was completely obliterated by newly formed bone deposits, the femur still retaining at this stage some of the original marrow cavity in a restricted region below the lesser trochanter. Obliteration of the femoral marrow cavity was not complete until oestrogen treatment had been carried out for at least 12 weeks. The osteogenic potentialities of both the oestrogenized male and female mice studied were apparently the same, there being no sexual differences in the degree of endosteal bone formed in response to hormone administration at any stage of the investigation.

Radiography of the undecalcified mouse skeleton in the later weeks of this investigation showed a graded response of various bones to oestrogen, the extent of medullary ossification diminishing in the same animal on comparing the tibia, femur, radius and ulna, humerus and finally the flat bones, in that order. No new bone formation was detected radiologically in the calvaria after 12 weeks of oestrogen administration.

Microradiography prior to decalcification of the hind limbs of mice injected intravascularly with radiopaque media, confirmed the advanced state of medullary bone accretion in the tibia and femur from the eighth week of treatment onwards (Pl. 1, figs. 1–3), besides demonstrating incidentally the vascularization of the soft tissues surrounding the bones. Comparison of control and experimental microradiographs, showed that the soft tissue arterial tree in the experimental hind limb underwent a pronounced vasodilatation.

Histological examination of normal and experimental material prepared from mice injected intravascularly with indian ink, yielded further information on the progress of osteogenesis in the oestrogen-treated animal. The growth cartilages persisted through the experimental period, completely separating epiphysis from metaphysis (Pl. 2, figs. 13–16). In the epiphyses of the oestrogen-treated mice, the juxta-epiphyseal plate of bone was thickened at an early period (2–4 weeks), bone thereafter being laid down on pre-existing spongy bone trabeculae and spreading inwards concentrically, encroaching on epiphyseal marrow areas (Pl. 2, figs. 13–16). After 8 weeks of treatment the epiphyseal marrow, in common with that of the shaft, was much reduced in quantity. In the metaphyses, the new bone likewise appeared to be laid down on and to extend from the existing spongiosa. Islands of new bone in the marrow were not encountered, although in thin histological sections bone spicules were seen leading the spread of metaphyseal bone deposition into the diaphysis.

Vascularization of diaphyseal marrow. Microangiography of 250 μ sections of mouse tibia and femur, taken from decalcified preparations following intravascular injection of radiopaque media, were particularly revealing when compared with normal microangiographs of these two bones. In both the normal and experimental animal, microangiography demonstrated in the diaphysis a wide central venous sinus related to the longitudinal axis of the bone, numerous fine medullary sinusoids draining into this structure, and small medullary arteries holding a generally straight course as they passed down through the substance of the marrow periphery. In particular, no essential difference was demonstrable between the normal and experimental microangiographs of tibial and femoral diaphyseal marrow examined after the 8th week of oestrogen administration (Pl. 1, figs. 4–9). Endosteal bone accretion was then known to be in an advanced state, as evidenced by the microradiographs of the undecalcified specimens prior to the taking of sections (Pl. 1, figs. 1–3). The vascular pattern in both the normal and experimental microangiographs was the same, judged by the relative disposition and form of the three major components of the vascular scaffolding of the marrow in the tibia and femur alike.

Histological examination of 15 μ sections cut from the same blocks that provided the diaphyseal microangiographs, as well as the histology of indian ink preparations, confirmed the fact that the medullary vascular scaffolding persists in spite of obliter-

ation of the marrow cavity by new bone. In the central portion of the diaphysis of the tibia following 8 weeks of oestrogen administration, the central venous sinus, medullary arteries and sinusoids could be seen engulfed by endosteal bone trabeculae. The newly formed bone was spongy in type and encroached on the blood forming areas of the marrow, but left intact the medullary vascular scaffolding as well as islands of haemopoiesis in the interstices of the trabeculae. After 12 weeks of oestrogen treatment, encroachment by endosteal bone on to the haemopoietic areas of the marrow had resulted in obliteration of the femoral cavity, where the components of the medullary vascular scaffolding were seen encased in bone (Pl. 1, figs. 10–12; Pl. 3, figs. 20–25).

Marrow vascularization in epiphyses and metaphyses. Microangiography of 250 μ sections, cut from the epiphyses and metaphyses of control and experimental mice, showed the type of vascularization found in bone extremities and gave some indication of relative vessel density in these regions. Large venous sinuses were prominent. Their tributaries, irregular in outline, were much more numerous than the small fine arterial terminals so that the vascular mesh in cancellous regions appeared largely venous in character (Pl. 2, figs. 17–19). In particular, control and experimental bone extremities were equally well vascularized, although the quantity of marrow in the oestrogen treated mice was known to have been considerably reduced (Pl. 2, figs. 15, 16).

The histology of indian ink preparations showed that in the epiphyses, fine arterial terminals and venous sinusoids passing peripherally towards the juxta-epiphyseal plate of bone and articular cartilage, together with more centrally placed large venous sinuses, were characteristic. In the metaphyses, large venous sinuses and numerous sinusoids, tortuous in course and irregular in outline, formed a dense vascular bed. Running through this were fine straight arterial terminals passing towards the growth cartilage where, in intimate contact with the cartilage columns, the finest arterial terminals suddenly formed hairpin loops which passed into juxta-epiphyseal sinusoids. In neither epiphyses nor metaphyses was there any evidence of arcade formation between small arterial twigs, nor were there any other signs of a functional anastomosis between the peripheral branches of the medullary arterial system, findings which suggested that the smaller arterial branches in bone marrow were end-arteries. Taken together, the histological and microangiographic records showed no essential difference in the vascular pattern, either in vessel type, disposition, or density, when the epiphyses and metaphyses of the control mice were compared with those of the oestrogen-treated animals (Pl. 2, figs. 13–19).

DISCUSSION

The results indicate that the response of immature mice treated with massive doses of oestradiol monobenzoate over a period of 3–15 weeks after birth includes a progressive development of endosteal new bone in the marrow cavities of the long bones. Obliteration of the tibial and femoral medullary cavities by new bone is achieved in 8 and 12 weeks respectively. Microangiographic and histological studies show that the blood vessels of the marrow persist, although the haemopoietic areas are replaced by new bone. In epiphysis, metaphysis and diaphysis the blood vessels

of the marrow in advanced states of medullary bone accretion maintain the same organization with respect to vessel type, density and relative disposition found in control animals.

It has already been emphasized that the dosage of oestrogen used in this experiment is highly unphysiological, but it is pointed out that endosteal bone formation has not yet been studied in the mouse when physiological doses of the order of 0·1 μg. per week have been administered. The dosage of oestradiol monobenzoate employed lies between the 0·1 mg. per week of Gardner & Pfeiffer (1938) and the 0·5–2·0 mg. per week of Urist *et al.* (1950), and has been chosen primarily to produce rapid endosteal bone formation as a pre-requisite to vascular studies on the bone marrow.

The graded response of the skeleton to oestrogen administration confirms in part the findings of previous workers (Urist *et al.* 1950), but the marrow of the tibia has been placed here before that of the femur in so far as its osteogenic potentiality is concerned. A graded medullary bone response to oestrogen treatment in the mouse skeleton is presumably due to possible inherent differences in osteogenic potency of marrow connective tissue cells according to their situation in the skeleton, or more probably to varying environmental circumstances such as oxygen potential, temperature, and pH in the different parts of the skeletal marrow. That bone marrow connective tissue cells, probably reticulum cells, have an inherent osteogenic potency not necessarily dependent on the presence of preformed bone, has been shown by Pfeiffer (1948) in her experimental transplants of femoral bone marrow to the testis and anterior chamber of the eye. Urist *et al.* (1948) have thought that oestrogen or one of its degradation products activated directly such undifferentiated medullary connective tissue cells to produce endosteal bone. On the other hand, it is interesting to note that Gardner (1943) suggests that the vascular supply of the myeloid and endosteal tissues is altered in oestrogenized mice, thus favouring osteoblastic proliferation and calcification. Silberberg & Silberberg (1941) consider that an increase in connective tissue around arterial terminals in the metaphyses of their oestrogenized mice might create a state of malnutrition favourable to bone formation. In oestrogenized birds, Landauer & Zondek (1944) have claimed that a heightened vascularization within the medulla is a prominent feature always, and have noted a thickening of the walls of some blood vessels and signs of vascular stasis. They conclude that endosteal bone formation represents a cicatrization of degenerating marrow.

No evidence of vascular occlusion, whether by fibrosis or endarteritic processes, which could lower medullary oxygen potentials, has been observed in the present investigation. On the contrary, the vascular organization of the marrow remains undisturbed in the presence of advanced medullary bone accretion. The findings also show that the vascular pattern of the marrow in mouse long bones, in the normal as well as the oestrogenized animal, is essentially similar to that described in the rabbit, rat, human foetus and adult man (Brookes & Harrison, 1957; Brookes, 1958*a*, *b*, 1960). Brookes (1958*a*, 1960) has offered visual evidence for regarding medullary arterial terminals as end-arteries, which the present investigation supports. It is concluded that there is a specific arrangement of blood vessels in bone marrow, which is applicable to mammals in general.

It is surprising that in the oestrogenized mouse the medullary vascular scaffolding

should persist unaltered, while bone is formed in a site where it is not normally present. The newly formed bone is spongy in type, reflecting the sinusoidal pattern of the normal mouse marrow, and not compact as might have been the case if the endosteal bone had borne no relation to the blood vessels of the marrow cavity on which it encroaches. It is difficult to conceive of bone formation occurring except in relationship to blood vessels, capillaries or sinusoids, which supply the raw materials for bone construction. Clinically, the presence of an adequate blood supply has long been held to be essential for callus and new bone production (Lacey, 1929). In embryonic life, the irruption of the vascular tuft and the removal of the cartilaginous model precedes bone formation in the shaft of the skeletal primordia. The perichondrial collar of bone, although forming before the irruption of the primary medullary blood vessels, nevertheless is laid down in relationship to the perichondrial capillaries which exist before the perichondrial osseous lamellae (Pinard, 1952). It is further pointed out that the vascular cartilage canals in young epiphyses are not haphazardly arranged, but have the same pattern as the arteries of the bony epiphyses which will replace them (Langer, 1876; Brookes, 1958*b*). It is difficult to see why epiphyseal vascular canal patterns in cartilaginous primordia should persist, in spite of subsequent osteogenesis, unless those patterns are specific for each epiphysis and determined by morphogenetic mechanisms, especially as the canal patterns are established long before a secondary centre of ossification appears. For these reasons, it is suggested that the vascular pattern of mammalian bone marrow is morphogenetically determined and is prior to and a major factor in the organization and arrangement of bone trabeculae which form in relationship to it.

SUMMARY

1. Immature mice were subjected to prolonged dosage with oestradiol monobenzoate in a 'split-litter' designed experiment. Femora and tibiae were examined at intervals microradiologically and histologically to observe the progress of medullary new bone formation and to elucidate the fate of the blood vessels in obliterated marrow regions.

2. The normal vascular patterns in epiphysis, metaphysis and diaphysis persisted despite extensive new bone formation which obliterated the haemopoietic elements.

3. Reasons are adduced for regarding the vascular pattern of mammalian bone marrow as morphogenetically determined, and a major factor in the organization of a trabecular pattern.

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EXPLANATION OF PLATES

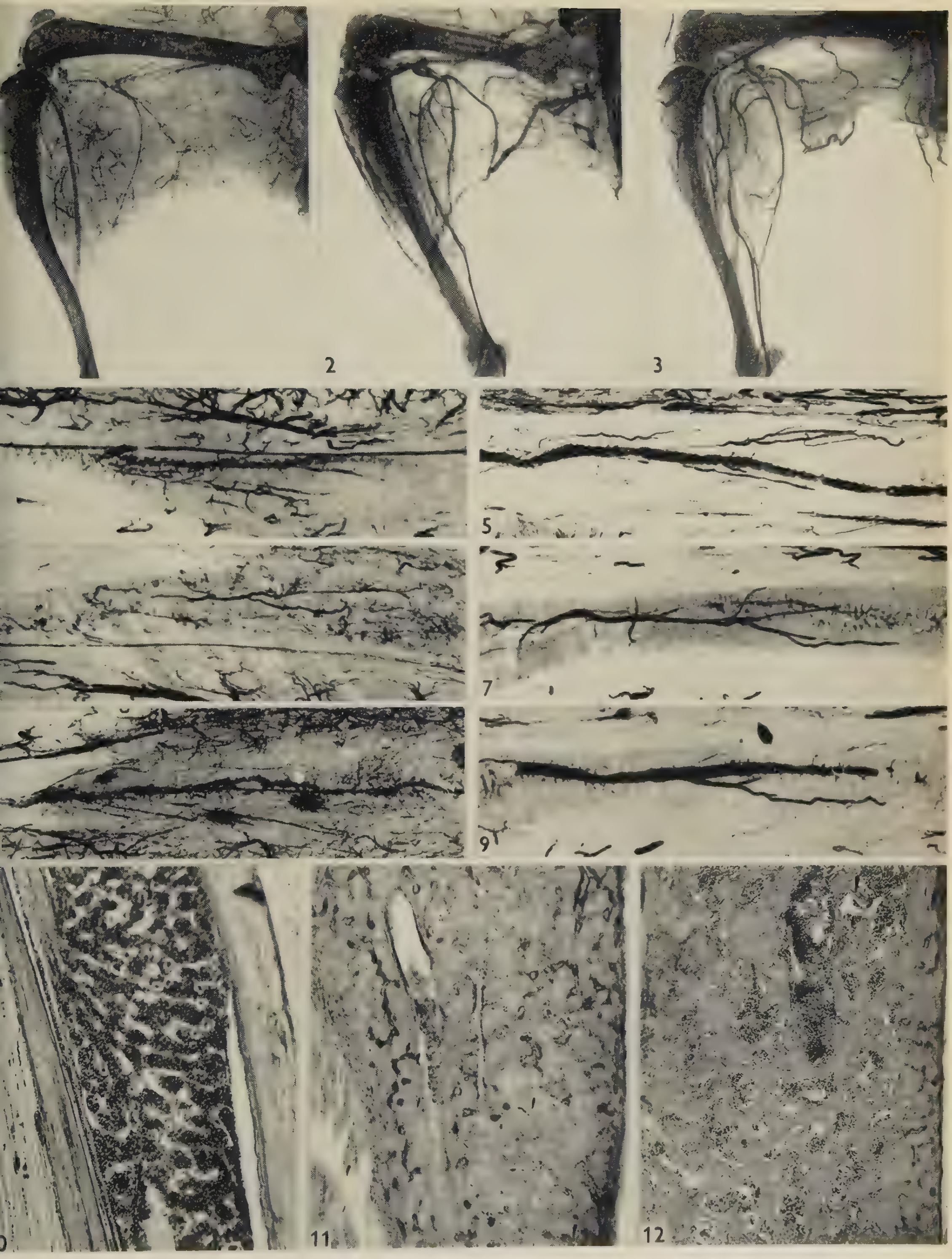
Plate 1

Fig. 1. Microradiograph of the hind limb of a 10-week-old control mouse showing the normal appearances of the undecalcified femur and tibia and the soft tissue arterial pattern. The specimen has been injected intravascularly with radiopaque media. ($\times 3{\cdot}5$).

Fig. 2. Microradiograph of a 10-week-old litter-mate of the animal used for fig. 1, after 8 weeks of oestrogen administration. The tibial marrow cavity has been obliterated and that of the femur nearly so by endosteal bone formation. The soft tissue blood vessels are dilated. ($\times 3{\cdot}5$.)

Fig. 3. Microradiograph of the undecalcified hind limb of a mouse after 12 weeks of oestrogen administration. Total obliteration of the femoral and tibial marrow cavities is complete. ($\times 3{\cdot}5$.)

Fig. 4. Control microangiograph of a 250μ sagittal section through the shaft of a 10-week-old



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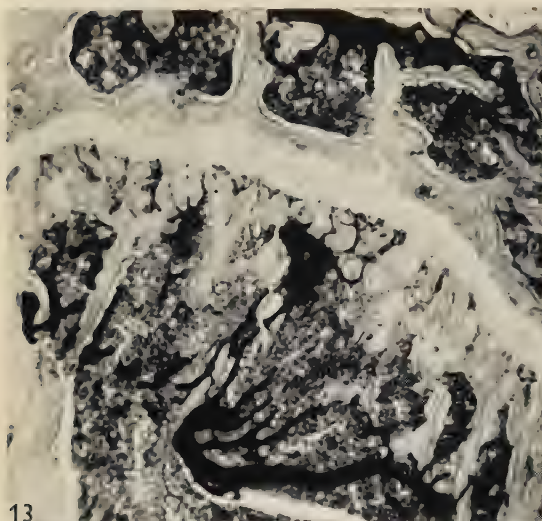
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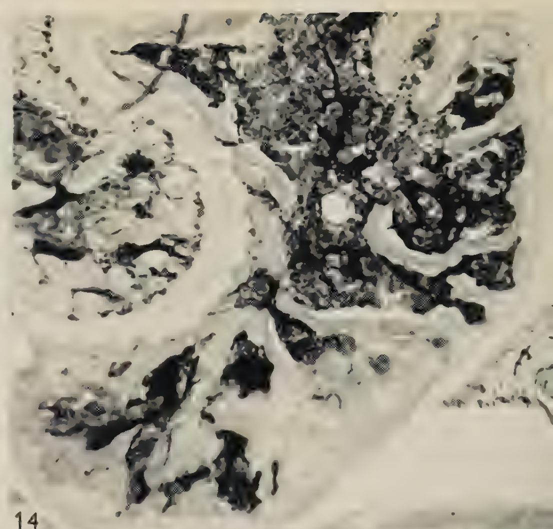
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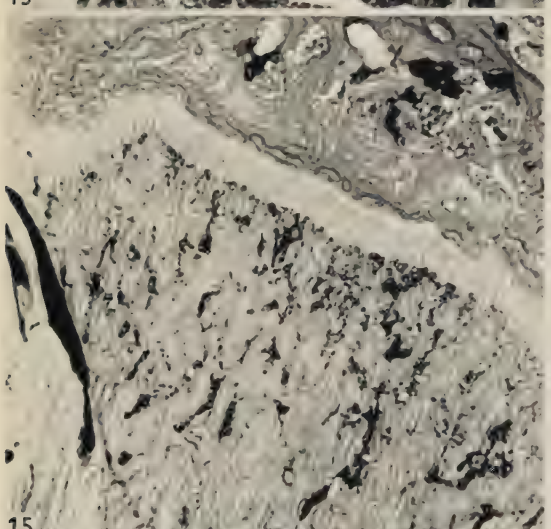
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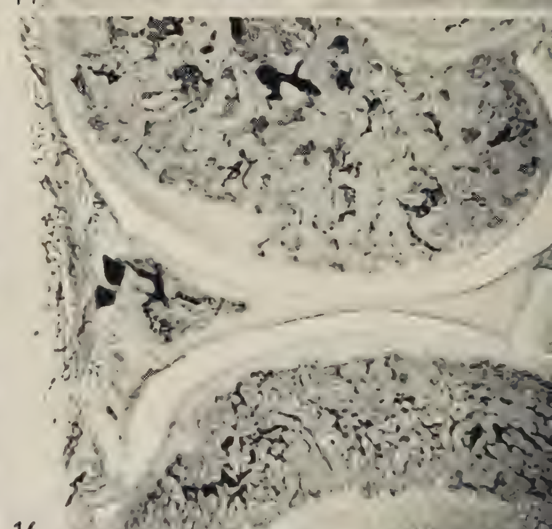
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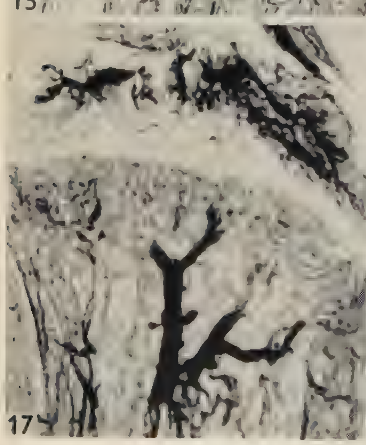
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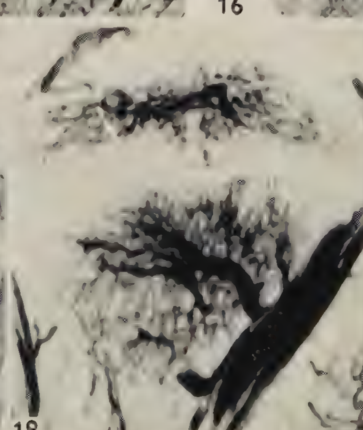
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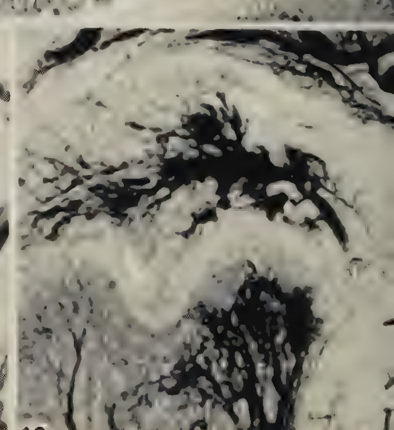
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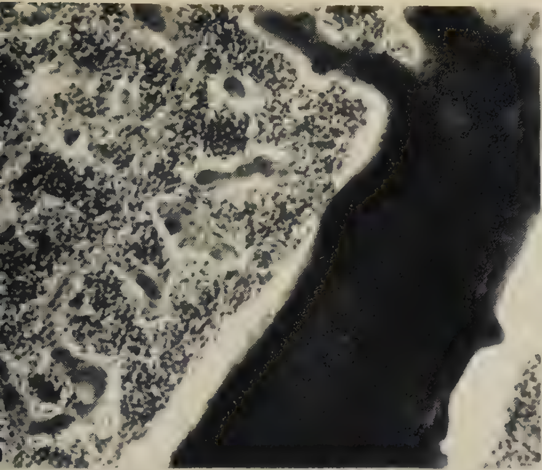

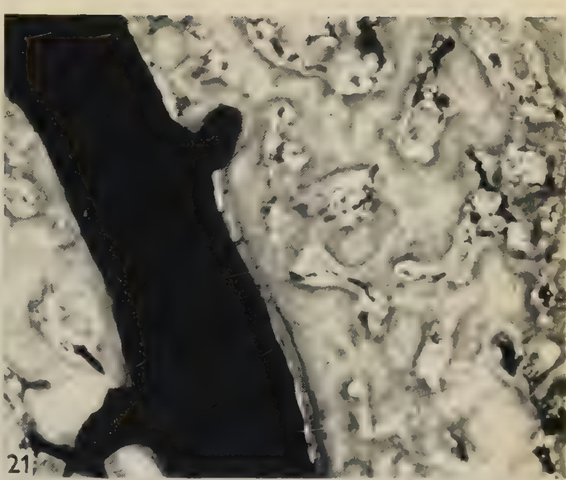

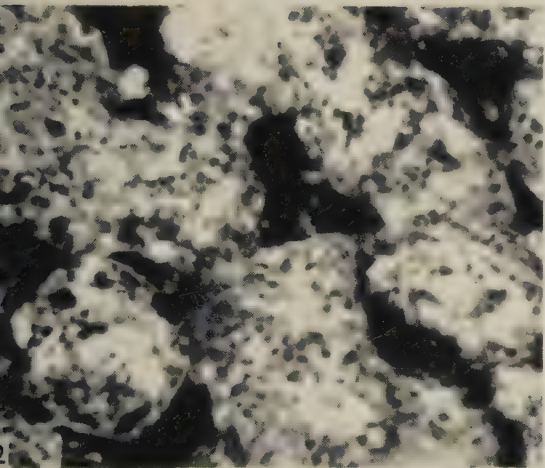

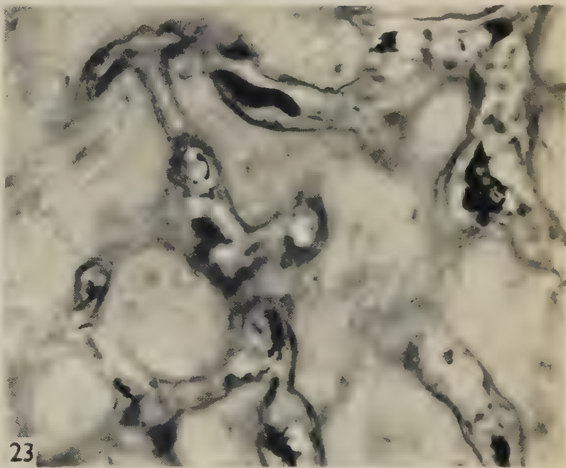

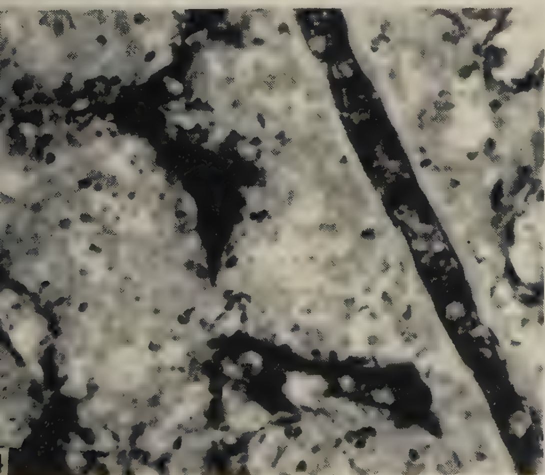

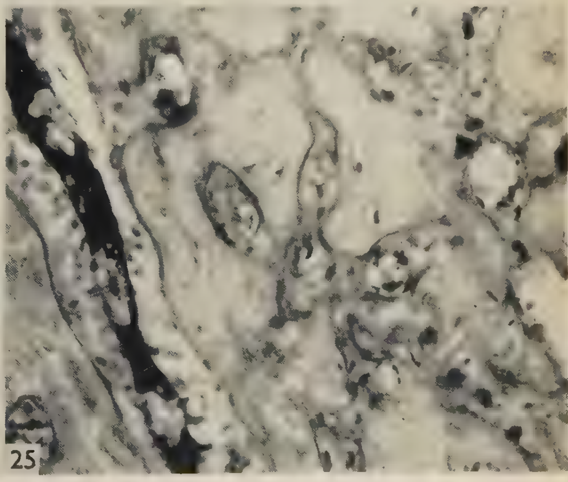

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mouse tibia. The break up of the principal nutrient artery, traversing the breadth of the picture, into ascending branches and a main descending one is seen, as well as a portion of the central venous sinus and medullary sinusoids. The diaphyseal marrow vessels occupy the central strip of the photograph, bounded above and below by the vessels of surrounding muscles. (×15.)

Fig. 5. Control microangiograph of a 250 μ sagittal section through the shaft of a 10-week-old mouse femur, showing the wide central venous sinus, medullary sinusoids, and fine medullary arteries coursing through the medulla. (×15.)

Fig. 6. Microangiograph of a 250 μ section of a litter-mate tibial diaphysis to the one shown in fig. 4, after 8 weeks oestrogen administration. The nutrient artery and its branches in the medulla are demonstrated with many medullary sinusoids. The central venous sinus was not included in the section. (×15.)

Fig. 7. Microangiograph of a 250 μ section of a mouse diaplyseal femoral marrow after 8 weeks oestrogen administration; litter-mate of fig. 5 mouse. Medullary branches of the principal nutrient artery are demonstrated, with medullary sinusoids joining the central venous sinus whose outlines only are visible. (×15.)

Fig. 8. Microangiograph of a 250 μ section of the tibial diaphysis of a mouse after 12 weeks oestrogen administration. Blood vessels of attached muscles are seen above and below in the illustration, and the marrow itself forms a strip across its breadth. The three main constituents of the medullary vascular scaffolding are demonstrated. (×15.)

Fig. 9. Microangiograph of a 250 μ section through the femoral diaphysis of a mouse after 12 weeks oestrogen administration. Medullary arteries, central venous sinuses and medullary sinusoids are demonstrated. (×15.)

Fig. 10. Control photomicrograph of 15 μ sagittal section through the tibia of the mouse shown in fig. 4. The principal nutrient canal can be seen in this illustration of normal mouse marrow. Haematoxylin and eosin. (×55.)

Fig. 11. Photomicrograph of 15 μ sagittal section through the central diaphysis of the tibia of the mouse shown in Fig. 6. The outlines of the central venous sinus, a medullary artery and numerous sinusoids are shown and demarcated by profuse endosteal bone production. Haematoxylin and eosin. (×55.)

Fig. 12. Photomicrograph of a 15 μ sagittal section through the central diaphysis of the femur shown in fig. 9. A portion of the central venous sinus and medullary sinusoids are shown encased in newly formed endosteal bone. Some haemopoietic tissue is still present. Haematoxylin and eosin. (×55.)

Plate 2

Fig. 13. Control photomicrograph of a 10 μ sagittal section through the upper tibial extremity of a 10-week-old mouse injected intravascularly with indian ink. Large venous sinuses in epiphysis and metaphysis are shown. A few cancelli and a normal growth cartilage are present. Haematoxylin and eosin. (×45.)

Fig. 14. Control photomicrograph of a 10 μ sagittal section through a 10-week-old mouse femoral condyle. Indian ink has been injected intravascularly. (×45.)

Fig. 15. Photomicrograph of a 10 μ sagittal section through the upper tibial extremity of a mouse, litter-mate to the one shown in fig. 13, after 8 weeks oestrogen administration. The growth cartilage is still present, and marrow sinusoids shown by the indian ink injection, are as numerous as the normal. Haemopoietic areas have been considerably encroached upon by endosteal bone. Haematoxylin and eosin. (×45.)

Fig. 16. Photomicrograph of a 10 μ sagittal section through the knee joint of a mouse given oestrogen for 10 weeks. Growth cartilages are still present but epiphyseal marrow has been very much reduced in volume. Epiphyseal vascularization as shown by intravascularly injected indian ink appears normal. Haematoxylin and eosin. (×45.)

Fig. 17. Control microangiograph of a 250 μ section through the upper portion of a 10-week old mouse tibia subsequent to intravascular injection of radiopaque media. The normal vascular appearances in epiphysis and metaphysis are shown. (×24.)

Fig. 18. Microangiograph of a 250 μ section through the upper tibial extremity of a mouse after 8 weeks oestrogen administration. The dense vascularization of epiphysis and metaphysis are demonstrated; a large metaphyseal vein is draining into the popliteal vein. (×24.)

Fig. 19. Microangiograph of a 250μ section through the femoral condyle of a mouse after 10 weeks oestrogen administration. Epiphyseal and metaphyseal vascularization is as dense as in the normal. ($\times 24$.)

PLATE 3

Fig. 20. Photomicrograph of a sagittal section through the femoral mid-diaphysis of a control mouse injected intravascularly with thorotrast solution. The normal appearances of the central venous sinus and medullary sinusoids embedded in haemopoietic tissue are shown. Haematoxylin and eosin. ($\times 155$.)

Fig. 21. Photomicrograph of a sagittal section through the femoral mid-diaphysis of a mouse injected intravascularly with indian ink after 12 weeks oestrogen treatment. The central venous sinus and medullary sinusoids are shown encased in new bone. Haematoxylin and eosin. ($\times 155$.)

Fig. 22. Control photomicrograph of mouse femoral diaphyseal marrow in which the sinusoids are shown injected with indian ink suspension. Haematoxylin and eosin. ($\times 400$.)

Fig. 23. Photomicrographs of mouse femoral diaphyseal bone marrow after 12 weeks oestrogen treatment: indian ink injected intravascularly. Sinusoids are shown encased in new bone formation which has almost entirely obliterated the haemopoietic areas. The pattern and density of the marrow sinusoids are unaltered. ($\times 400$.)

Fig. 24. Control photomicrograph of a mid-diaphyseal tibial bone marrow from a mouse injected intravascularly with indian ink, showing a typical straight thin-walled artery passing through haemopoietic tissue and sinusoids. Haematoxylin and eosin. ($\times 400$.)

Fig. 25. Photomicrograph of mouse tibial mid-diaphyseal marrow after 8 weeks oestrogen treatment. A typical fine medullary artery and sinusoids are shown engulfed in new bone, which has almost obliterated the haemopoietic spaces leaving the marrow vessels intact. Haematoxylin and eosin. ($\times 400$.)

CONNEXIONS OF THE DORSAL TEGMENTAL NUCLEUS IN RAT AND RABBIT

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INTRODUCTION

The dorsal tegmental nucleus of Gudden (1889) forms a continuation of the central grey of the midbrain in the floor of the fourth ventricle. The relationship of the nucleus to the fibres of the dorsal longitudinal fasciculus is notable, for this fasciculus is thought to convey impulses between the hypothalamus and the bulbar efferent centres (Ariëns Kappers, Huber & Crosby, 1936, p. 593). Although many connexions have been proposed for the dorsal longitudinal fasciculus, few have been confirmed experimentally, nor has the extent of dorsal tegmental participation in this fibre system been precisely determined.

Efferent or afferent connexions of the dorsal tegmental nucleus with the mamillary peduncle were suggested by Koelliker (1896), Déjerine (1901), and Castaldi (1923). Investigations with the Marchi method by Probst (1902) and Bodian (1940) have demonstrated only afferent fibres in the mamillary peduncle to the mamillary body. Akert & Andy (1955) and Guillery (1956) have provided evidence that the dorsal tegmental nucleus may contribute to this projection. The high degree of organization of the connexions of the mamillothalamic system is well documented (Powell, 1958), but it is not known precisely how dorsal tegmental projections may be involved in this system.

The present study establishes that the dorsal tegmental nucleus contributes to the mamillary peduncle and reveals the organization of this projection with respect to the mamillary body and medial forebrain bundle. Evidence is also provided that the dorsal tegmental nucleus is a major synaptic station for the dorsal longitudinal pathways, some of which are elucidated.

MATERIALS AND METHODS

The data were derived from study of eleven rabbits and eight rats. Electrolytic lesions were made in or near the dorsal tegmental nucleus of rabbits under pento-barbitone anaesthesia with the tips of needle electrodes mounted in a stereotaxic device.† Control lesions were placed in the collicular sites traversed by the electrodes in making dorsal tegmental lesions and in the cerebellum and medial vestibular nucleus. Lesions were made in rats under ether anaesthesia by inserting fine curved needles through the foramen magnum and under the cerebellum to the appropriate level of the brain stem. Brains with damage to the brachium con-

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† In some rabbits the lesion site was first stimulated, whilst respiration and blood pressure were recorded by Dr B. G. Cragg (1959).

junctivum were not included in the study. The rabbits were kept alive 7–11 and the rats 5–8 days before perfusion with 10% formol saline. The brain stem and cerebellum were fixed in 10% formol saline for 1–6 months before sagittal frozen sections were cut at 20–25 μ. The extent of a lesion was examined in Nissl-stained sections and fibre degeneration in sections impregnated by the Nauta–Gygax method (Nauta & Gygax, 1954) or by a Laidlaw modification of it (Chambers, Liu & Liu, 1956). The unoperated brains of two rats and two rabbits were simultaneously impregnated with the experimental sections for histological controls.

Since the findings involve several regions of the brain in two species, it has been necessary to give considerable attention to control material and to criteria for evaluating degeneration. The appearance of fibres impregnated by the Nauta methods depends on many factors, including the post-operative survival time of the animal, period of fixation, thickness of the sections, exact composition of the solutions, and the times that the sections remain in each solution. Variations among species may be expected to influence the time-course of degeneration. In the present study a longer survival was required to obtain maximum degeneration in the rabbits than in the rats. In general, only fibres that were clearly broken up into irregular droplets and granules, that could be traced from the lesion, and that could not be matched in control materials were finally accepted as degenerating. In appraising the degeneration it was appreciated that fine fibres may disintegrate later than coarse ones (Van Crevel, 1958).

It has been expedient to describe the intensity of degeneration in certain regions, since the intensity often bore a strong correlation with the location of the lesion. Although the apparent intensity may be influenced by any of the factors mentioned above, such factors can be compensated by limiting assessments to optimally impregnated sections at the stage of maximal degeneration. 'Massive', 'dense', 'moderate', and 'sparse' have been used to describe, in that order, decreasing degrees of relative intensity (Pl. 2; Pl. 3, fig. 9). Likewise, 'coarse', 'medium', and 'fine' have been used to describe the relative calibres of degenerating fibres, which in many cases were correlated with their relative sizes in normal material impregnated with the silver method of Holmes (1942).

The terminology of Meessen & Olszewski (1949) is used for the rhombencephalon, of Gillilan (1943) for the mesencephalon, and of Gurdjian (1927) for the diencephalon of both rat and rabbit. For the subdivisions of the dorsal tegmental nucleus a scheme is used which has been suggested by Guillery & Powell. (These workers (unpublished) find, after mamillary lesions in the rat, retrograde degeneration confined to partes centralis and ventromedialis.) The subdivisions are called partes centralis, ventromedialis, anterior, and posterior (Pl. 1, fig. 1). Pars centralis corresponds to nucleus *q* of Meessen & Olszewski and consists of medium-sized cells densely infiltrated with a plexus of fine fibres. Ventromedial to it and more posterior is pars ventromedialis, a small group of medium-sized cells near the median raphé. Pars anterior and pars posterior are the anterior and posterior extensions, respectively, of pars centralis, but they contain smaller, less densely packed cells. Surrounding the dorsal tegmental nucleus are loosely arranged, very small cells, resembling the undifferentiated portions of the central grey.

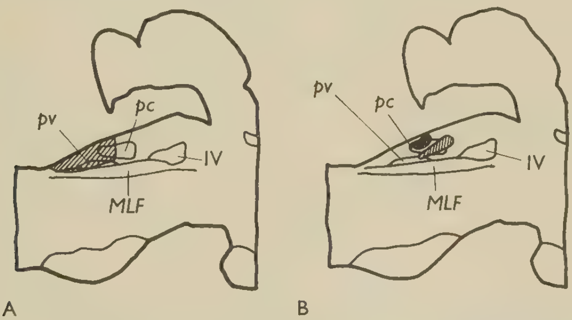
RESULTS

The findings are presented in three parts, anterior connexions, posterior connexions, and control lesions. All the lesions and resulting degeneration are ipsilateral unless otherwise stated.

(1) *Anterior connexions*

(*a*) *Mamillary peduncle*

General observations. Lesions in the dorsal tegmental nucleus produce extensive degeneration in the mamillary peduncle (Pl. 1, fig. 2). There is a correlation between destruction of pars centralis and degeneration in the medial mamillary nucleus and between destruction of pars ventromedialis and degeneration in the lateral mamillary nucleus. Degenerated fibres leaving the mamillary peduncle appear in the medial forebrain bundle and nucleus of the diagonal band of Broca and reach the medial septal nucleus in the diagonal band. In association with the above there is degeneration in the ventral tegmental area of Tsai and the posterior hypothalamic nucleus. Preterminal degeneration also appears in nucleus medialis profundus, centralis superior, and the tegmental reticular nucleus.

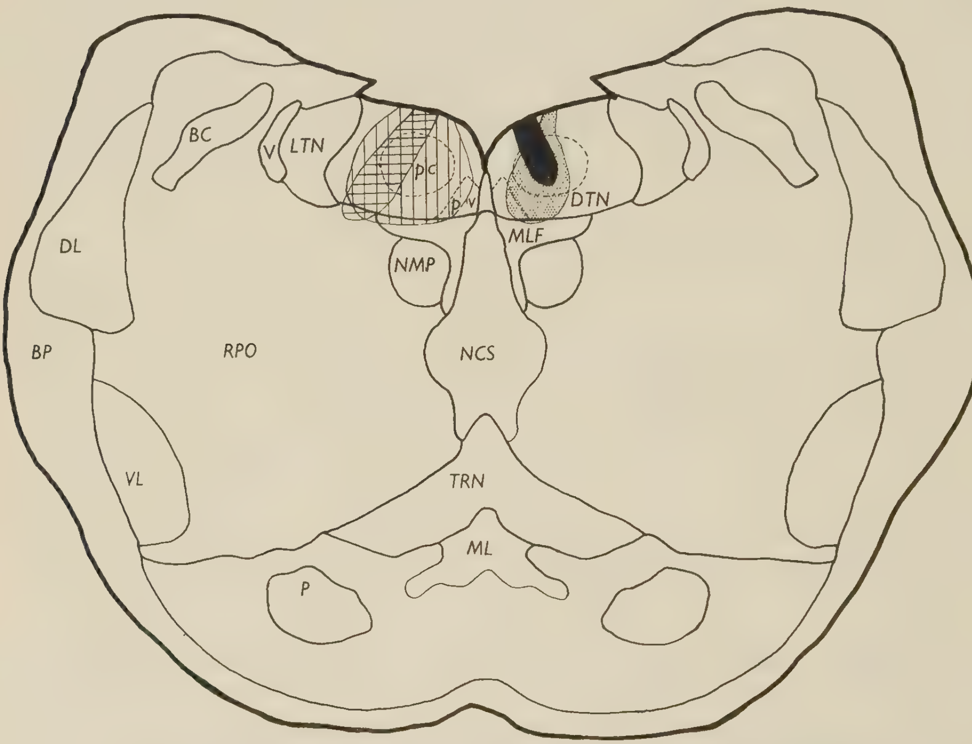


Text-fig. 1. Parasagittal diagram of the maximum extents of the lesions in A, rabbit T2; B, rabbit T7 (black), DT1 (hatched).

Rabbits. In rabbit T2 (Text-figs. 1A, 2) all parts of the dorsal tegmental nucleus are damaged except the anterior half of pars anterior. Massive degeneration of coarse and medium fibres streams anteroventrally through nucleus centralis superior, nucleus medialis profundus (ventral or deep tegmental nucleus of Gudden), and the tegmental reticular nucleus of Bechterew ('Ppl' of Meessen & Olszewski). More laterally there is only sparse degeneration in the reticular formation, whilst in the interpeduncular region the degeneration turns anteriorly into the mamillary peduncle. Degeneration passes through the nuclear groups mentioned in unreduced intensity, although fine degenerated fibres coil about the cells. The coarse degeneration of the mamillary peduncle ends massively in the lateral mamillary nucleus. Medium-sized fibres form a dense nest of degeneration in nucleus premamillaris dorsalis and the medial mamillary nucleus, chiefly in pars medianus* but also anteriorly in pars medialis. Medium-sized degenerated fibres, reduced in

* Although Cowan & Powell (1954) do not delineate in the rabbit the homology of pars medianus of the rat, the term is used to facilitate comparison between these species.

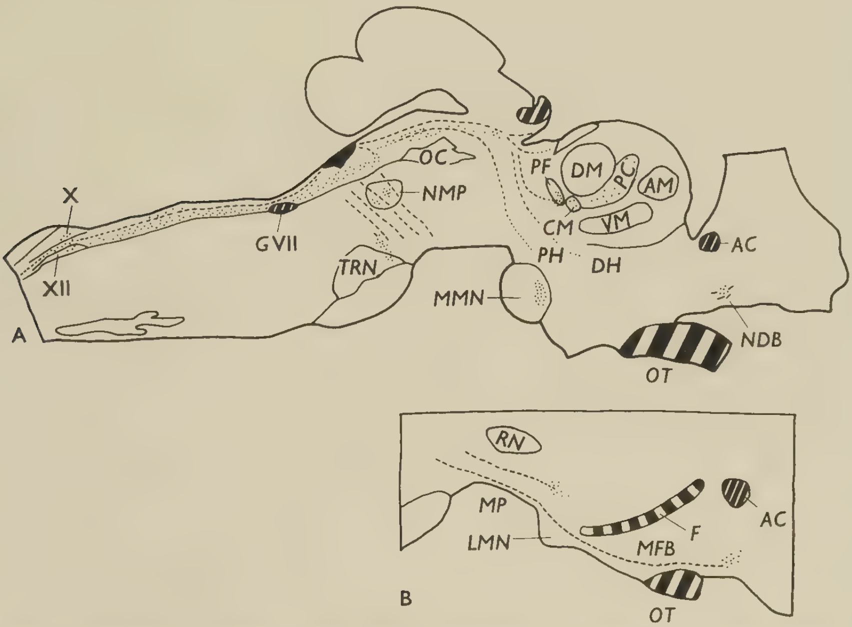
number, can be traced from the mamillary peduncle into the medial forebrain bundle. Decreasing in intensity, they ascend to the lateral preoptic area, and, forming moderate degeneration in the nucleus of the diagonal band, disappear in the region of the medial septal nucleus. At the posterior end of the interpeduncular region a moderate number of degenerated fibres take a course dorsal to, and separate from, the mamillary peduncle and ascend through the ventral tegmental area of Tsai. These medium-sized fibres form a knot of degeneration in the region of the supramamillary decussation. Some of them continue forward into the posterior hypothalamic nucleus and laterally may contribute to the degeneration in the medial forebrain bundle.



Text-fig. 2. Lesions in rabbits T2 (vertical shading), DT3 (horizontal shading), T8 (black), and DT1 (stippling) reconstructed in a transverse plane.

The lesion in rabbit T1 is similar to that in T2 but larger, and the degeneration of the mamillary peduncle follows the same pattern. In rabbit DT1 the lesion (Text-figs. 1B, 2) destroys pars anterior of the dorsal tegmental nucleus and damages partes centralis and ventromedialis; degeneration appears in both the medial and lateral mamillary nuclei. In rabbit DT3 the lesion (Text-fig. 2) lies more anterior than that in DT1 and involves pars centralis, but not pars ventromedialis; the mamillary degeneration is limited to the medial nucleus. The degeneration takes a more anterior course and does not appear in the tegmental reticular nucleus. In DT1 there is a small nest of degeneration in the anterior end of the inter-

peduncular nucleus. Otherwise the pattern of degeneration in both DT1 and DT3 (Pl. 2) resembles that in rabbit T2. In rabbits T7 and T8 the lesions (Text-figs. 1B, 2) involve only the dorsal undifferentiated grey and pars centralis. In both animals the degeneration follows the same pattern as in rabbit T2 but does not appear in the lateral mamillary nucleus (Text-fig. 3). In rabbit DT2 the dorsal tegmental nucleus and the reticular formation posterior to nucleus medialis profundus are destroyed. Transverse sections were made to confirm that the degenerated fibres pass ventrally near the midline until, near the base of the brain, they turn laterally into the mamillary peduncle. There is degeneration in the mamillary body and medial forebrain bundle as in rabbit T2.



Text-fig. 3. Parasagittal drawing of lesion and degeneration in rabbit T8. A, Degeneration in the *dlf* descends dorsal to *G*VII, ascends dorsal to *OC*. B, A more lateral plane. Note degeneration in the ventral tegmental area of Tsai dorsal to that in *MP*. Notes: The isthmus and floor of the fourth ventricle are expanded for clarity. Dots indicate preterminal degeneration; dashes, fibres of passage (not necessarily excluding terminals along their course). All degeneration is ipsilateral to the lesion.

Rats. The lesion in rat 484 involves partes ventromedialis and posterior but spares pars centralis (Text-figs. 4, 5). There is also damage to the posterodorsal portion of nucleus prepositus hypoglossi and to nucleus recessus incertus (Chatfield & Lyman, 1954). The ventral mesencephalic degeneration is the same as in the rabbits. Degeneration in the mamillary body is limited to the lateral mamillary nucleus; no degeneration occurs at more anterior levels. In rat 480 the lesion is limited to partes posterior and centralis, ipsilaterally, and to pars ventromedialis, bilaterally (Text-figs. 4, 5). Degeneration occurs in the medial mamillary nucleus, ipsilaterally, and in the lateral mamillary nucleus, bilaterally. Degeneration enters the ipsi-

lateral medial forebrain bundle, lateral preoptic area, and nucleus of the diagonal band.

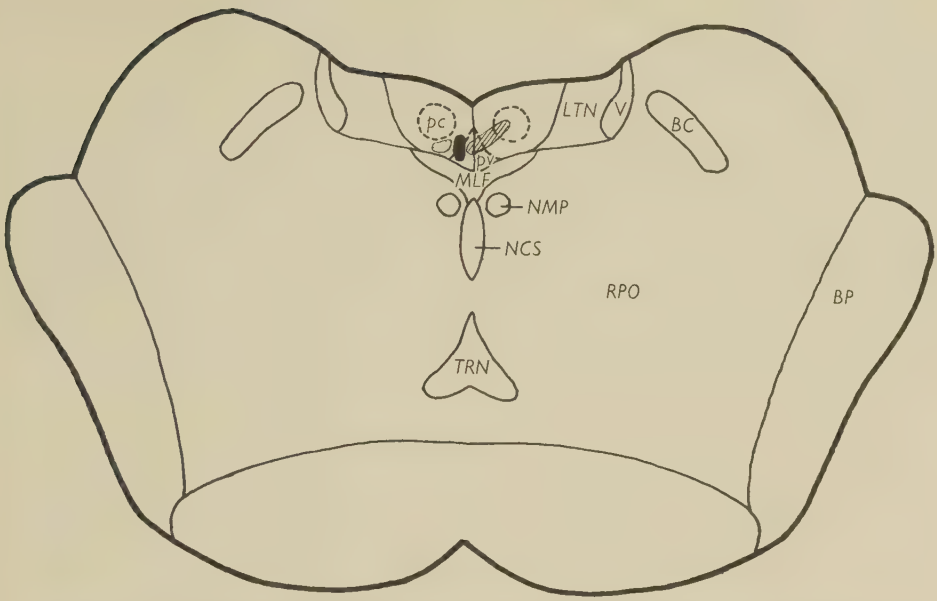
In rat 478 there is damage bilaterally to the posterior floor of the fourth ventricle and unilateral undercutting of pars centralis and damage to pars posterior but not to pars ventromedialis (Text-fig. 4). Transverse sections were made to obtain optimum representation of the medial septal nucleus. Degeneration, ipsilateral to the dorsal tegmental lesion only, passes in the mamillary peduncle to partes medianus and medialis of the medial, but not the lateral, mamillary nucleus. Degenerated fibres ascend in the medial forebrain bundle and through the diagonal band to the medial septal nucleus. A few fine degenerated fibres turn dorsomedially from the mamillary peduncle to ascend beside the habenulo-peduncular tract to the dorsomedial nucleus of the thalamus.

(*b*) *Dorsal longitudinal fasciculus* (*dlf*)

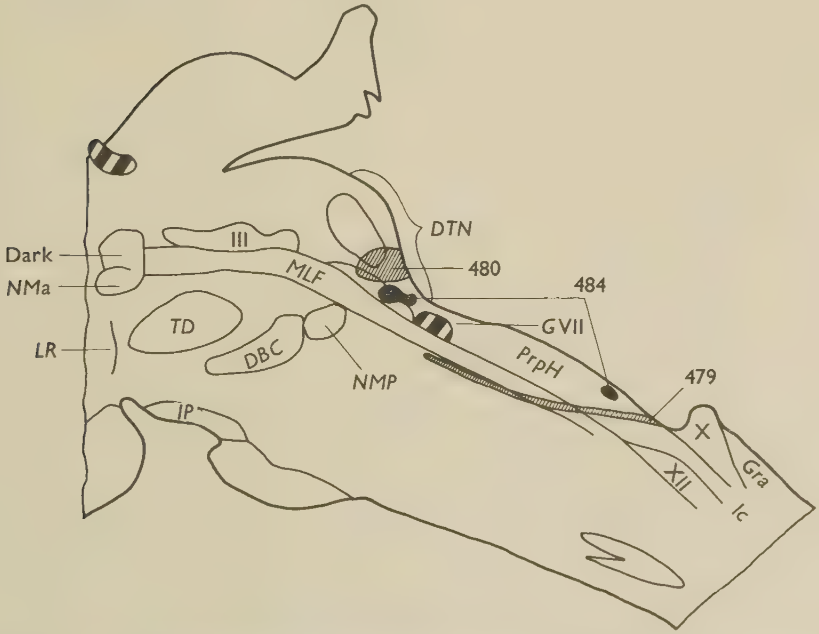
General observations. Lesions in the anterior half of the dorsal tegmental nucleus produce numerous degenerated fibres in the *dlf* supplying the central grey and several midline mesencephalic nuclei. A few such fibres reach the pretectal nucleus, the intralaminar and dorsomedial nuclei of the thalamus, and the posterior and dorsal hypothalamus. Unlike the degeneration in the mamillary peduncle, that in the *dlf* is diffuse, progressively diminishes in intensity, and reflects the presence of multiple synapses. Significant degeneration in the mesencephalic *dlf* does not descend posterior to the dorsal tegmental nucleus. The pattern of degeneration following complete destruction of the mesencephalic *dlf* resembles that following dorsal tegmental lesions but is more extensive and considerably denser.

Ascending fibres. In rabbits T7 and T8 the lesion completely spares the medial longitudinal fasciculus ('*flp*' of Meessen & Olszewski) and nearby tegmental fibres (Text-figs. 1–3). Fine degenerated fibres ascend in the ventral and lateral parts of the central grey (*dlf*). They reach the nucleus of Darkschewitsch and nuclei medialis anterior and linea rostralis anterior to the oculomotor complex and then turn ventrally to merge with the degeneration already described in the ventral tegmental area of Tsai and the posterior hypothalamic nucleus. Other fine fibres enter the pretectal nucleus, whilst some turn ventrally in the periventricular system to the nuclei centrum medianum, parafascicularis, and centralis and, very sparsely, to the posterior paraventricular, ventromedial, and dorsomedial nuclei of the thalamus. The remnant of the periventricular degeneration disappears in the dorsal portion of the posterior hypothalamic nucleus and the posterior region of the dorsal hypothalamic area (Rioch, Wislocki & O'Leary, 1940). In the posterior midbrain the degeneration in the *dlf* is dense, but its intensity progressively diminishes to moderate or sparse in the diencephalon. The degeneration in the other rabbits is similar to the above but more or less intense according to the size of the lesion. In rabbit DT1 with only minor damage to the medial longitudinal fasciculus, degenerated fibres pass from the *dlf* (Pl. 3, fig. 9) to the dorsal nucleus of the raphé and ventrally through the caudal linear grey* to the ventral tegmental area of Tsai. In rabbits T2 and DT3 damaged tegmental fibres near the medial longitudinal

* Nucleus linea caudalis, as so labelled in Gillilan's fig. 5 (1943) but not as in other papers of the same series.



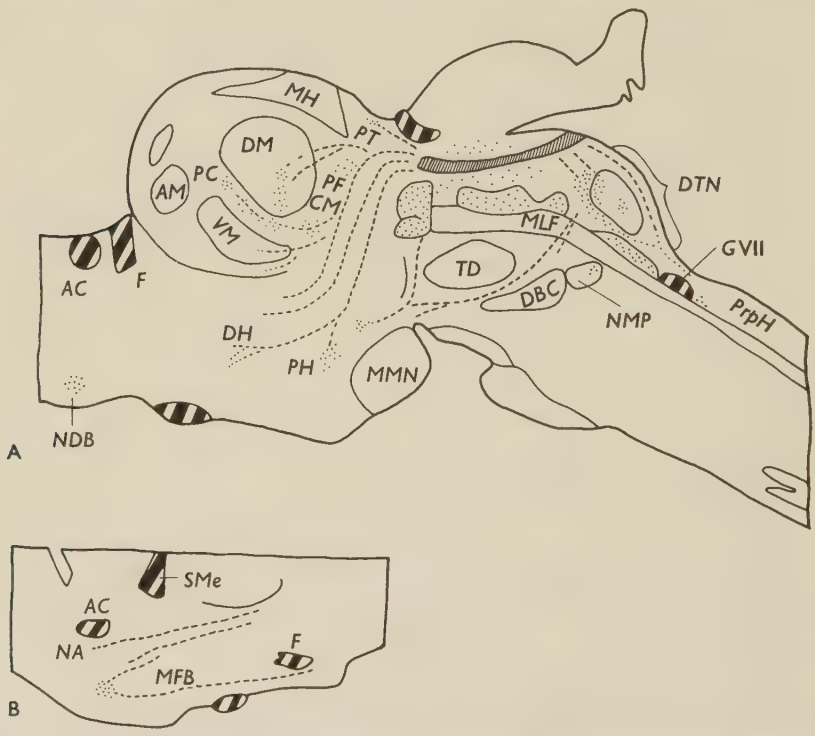
Text-fig. 4. The lesions in rats 480 (diagonal shading), 484 (black), and 478 (stippling) reconstructed in a transverse plane.



Text-fig. 5. Parasagittal drawing of lesions in rats 479, 480, 484. Nucleus prepositus hypoglossi and the dorsal tegmental nucleus are expanded. Nucleus medialis profundus and the facial genu are lateral to the plane of the diagram, but their relative positions are shown.

fasciculus have caused medium and coarse degeneration in several of the thalamic intralaminar nuclei. In DT3 very fine periventricular degeneration of moderate intensity reaches pars dorsalis of the posterior periventricular nucleus of the hypothalamus.

Descending fibres. In rabbit DT1 (Text-fig. 1B) dense degeneration appears throughout the dorsal tegmental nucleus, but only very sparse degeneration passes over the facial genu to the anteroventral portion of nucleus prepositus hypoglossi. The more anterior lesion in rabbit DT3 has caused dense degeneration in all parts of the dorsal tegmental nucleus, especially in its anterior half, but none posterior to the facial genu. There is sparse degeneration in the laterodorsal tegmental nucleus, or locus coeruleus, of both rabbits. The paucity of degeneration posterior to the facial genu in these rabbits contrasts with those in which the lesion involves more posterior portions of the dorsal tegmental nucleus (see rabbit T8 below).



Text-fig. 6. A, Lesion (hatching) and degeneration in rat 474. Note degeneration passing through rostral and caudal linear grey, anterior and posterior, respectively, to *TD*. B, A more lateral plane. For notes see Text-fig. 5.

Rats. In rat 480, with a lesion involving the posterior third of pars centralis, sparse degeneration ascends the *dlf*; a few fibres reach nucleus parafascicularis in the diencephalon. In rat 484, in which the dorsal tegmental lesion is more posterior, and in rats 481 (Text-fig. 7) and 479 (Text-fig. 5), with lesions posterior to the dorsal tegmental nucleus, there is no degeneration in the mesencephalic central grey.

In rat 474 a lesion extends through the entire length of the mesencephalic central grey on one side. It involves neither the tegmental tracts peripherally nor the

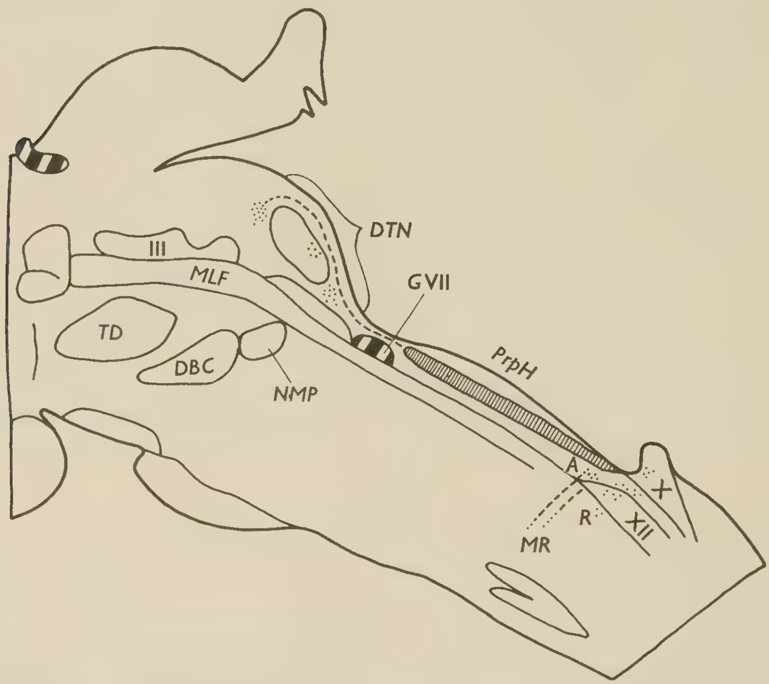
oculomotor complex or dorsal tegmental nucleus but reaches the posteroventral edge of the posterior commissure (Text-fig. 6). The *dlf* is massively degenerated (Pl. 3, fig. 11) and distributes in the midbrain and thalamus essentially as in the rabbits with dorsal tegmental lesions, but considerably more intensely. Degenerated fibres reach all the intralaminar nuclei, including nuclei centralis, paracentralis, and centralis lateralis (lateral part of Gurdjian's paracentralis). Most of the remaining dense periventricular degeneration ends in the posterior hypothalamic nucleus and the dorsal hypothalamic area. A few degenerated fibres reach pars dorsalis of the posterior periventricular nucleus, but none occur further ventrally or in the ventromedial, dorsomedial, filiform, or supraoptic nuclei. Some of the fibres enter the medial forebrain bundle anteriorly, in which degeneration ascends to the nucleus of the diagonal band. More laterally (Text-fig. 6B) degeneration ascends just ventral to the thalamus to the nucleus accumbens and the region ventral to the anterior commissure and anterior to the stria medullaris. Finally, degenerated fibres from the central grey course in moderate intensity through the caudal, intermediate, and rostral linear grey (nuclear groups of Gillilan, 1943) and through the ventral tegmental area of Tsai to the supramamillary region and the medial part of the medial forebrain bundle. Degeneration elsewhere in the reticular formation and in the superior colliculus seems sparse in the sagittal sections used. Descending degeneration reaches all parts of the dorsal tegmental nucleus (dense in pars anterior, moderate in partes posterior and ventromedialis, moderate to sparse in pars centralis) and nucleus recessus incertus. A few degenerated fibres go to the laterodorsal tegmental nucleus and to nuclei medialis profundus and centralis superior. Only sparse degeneration occurs posterior to the facial genu in nucleus prepositus hypoglossi and the medial vestibular nucleus ('Trg' of Meessen & Olszewski).

(2) *Posterior connexions*

Efferent pathways. The lesion in rabbit T8 has produced very fine degenerated fibres passing posteriorly over the facial genu densely into nucleus prepositus hypoglossi (Pl. 3, fig. 12) and sparsely into the anterodorsal region of the medial vestibular nucleus. The intensity of the degeneration diminishes as it descends through nucleus prepositus hypoglossi and, after supplying pars gk 25 (Meessen & Olszewski) just anterior to the hypoglossal nucleus rather densely, enters nucleus intercalatus with less intensity (Text-fig. 3). There is sparse degeneration of very fine fibres in the ventral portion of the dorsal motor vagal nucleus ('Al' of Meessen & Olszewski) and in the dorsal portion of the hypoglossal nucleus and questionable degeneration in pars parvocellularis of nucleus tractus solitarius. Horizontal sections of the upper cervical cord reveal the remnant of the degeneration descending lateral, then ventrolateral, to the central canal. In rabbits T2 and T7 (Text-fig. 1) the pattern is similar, but sparse, medium and coarse degeneration from the medial longitudinal fasciculus of rabbit T2 enters the abducens, vestibular, and hypoglossal nuclei.

In rat 480 (Text-fig. 5) fine degenerated fibres pass over the facial genu into nucleus prepositus hypoglossi. No degenerated fibres seem to reach nucleus intercalatus, but there is sparse degeneration in the posterior nucleus tractus solitarius bilaterally.

Afferent pathways. In rat 481 the lesion is limited entirely to nucleus prepositus hypoglossi (Text-fig. 7). Fine fibres ascend densely over the facial genu to form a nest of degeneration in pars posterior of the dorsal tegmental nucleus (Pl. 3, fig. 13). Other fibres continue anterodorsally to partes anterior and centralis. In rat 479 (Text-fig. 5) fine degenerated fibres occur throughout nucleus prepositus hypoglossi and ascend over the facial genu to pars posterior and the posterior half of pars centralis. In rats 478 and 484 (Text-fig. 5), with damage to the floor of the fourth ventricle, there is dense degeneration throughout the dorsal tegmental nucleus. In none of these animals is there degeneration in the central grey anterior to the dorsal tegmental nucleus.



Text-fig. 7. The lesion and degeneration in rat 481. Degeneration in nucleus prepositus hypoglossi is largely omitted. For notes see Text-fig. 5.

Nucleus prepositus hypoglossi. The lesions in rats 479, 481, and 484 destroyed, besides cells in nucleus prepositus hypoglossi, fibres of passage from the dorsal tegmental nucleus. Consequently degeneration occurs in the same regions directly supplied by the latter nucleus, but additional features are present. In each animal a prominent stream of degenerated fibres passes from pars gk 25 of nucleus prepositus hypoglossi into the medial reticular formation (Text-fig. 7). In the same rats careful examination suggests that the descending fibres enter the hypoglossal nucleus directly at its anterior pole, a few fibres passing to the nucleus of Roller. Degenerating fibres enter the dorsal motor vagal nucleus along its ventral margin from nucleus intercalatus.

(3) *Control lesions*

In rabbits with very small collicular lesions degenerated fibres pass in a ventrolaterally directed arc to the cuneiform area but not to any of the structures previously considered except the colliculi. In one rabbit there is a necrotic area on the cerebellar pyramis but no degeneration in the dorsal tegmental nucleus. In another there is extensive damage to the vermis and roof nuclei of the cerebellum and a small lesion in the medial vestibular nucleus. In this animal medium-sized degenerating fibres pass into nucleus prepositus hypoglossi and sparsely into nucleus intercalatus. Degeneration is also present in the laterodorsal tegmental nucleus, the medial longitudinal fasciculus and abducens nucleus, the brachium conjunctivum and the pontine reticular formation. There is no degeneration in the dorsal tegmental nucleus or supragenual grey nor in the vagal or hypoglossal nuclei. This material shows that degeneration arising from the electrode tracks did not contribute to the degeneration previously described and that the dorsal tegmental lesions themselves probably did not interrupt fibres of passage from the cerebellum or medial vestibular nucleus.

DISCUSSION

First, the origin and distribution of the mamillary peduncle will be discussed; second, the dorsal tegmental connexions with certain paramedian nuclei of the pons and midbrain; and third, the relation of the dorsal tegmental nucleus to the *dlf* and its connexions.

(1) *Mamillary peduncle*

Origin. The Marchi studies of Probst (1902), Bodian (1940), and Morin (1950) have failed to support the contention that the dorsal tegmental nucleus is a terminal site of mamillary peduncle fibres (Koelliker, 1896). Instead the present study demonstrates that the dorsal tegmental nucleus is a major source of the mamillary peduncle, as suggested by Guillery (1956) in the rat and Akert & Andy (1955) in the cat. Evidence that nucleus medialis profundus contributes to the mamillary peduncle has been provided by retrograde degeneration studies in the cat (Fox, 1941; Akert & Andy, 1955). There is no conclusive evidence that the nucleus of the mamillary peduncle (Papez, 1932) contributes to this tract, although retrograde degeneration has been observed in this nucleus following posterior hypothalamic lesions (Fox, 1941). Ramón y Cajal (1955, p. 461) and Wallenberg (1899) have sought a contribution from the medial lemniscus, but numerous investigations do not support such a proposal (e.g. Ranson & Ingram, 1932). The possibility that the tegmental reticular nucleus and nucleus centralis superior may contribute should be considered, for these nuclei are associated with the tegmental fibres to the mamillary peduncle and from the mamillary (Guillery, 1957) and limbic (Nauta, 1958) projection sites of the peduncle.

Distribution. Gudden (1880) observed the connexion between the mamillary peduncle and the lateral mamillary nucleus, and Wallenberg (1899), using the Marchi method, demonstrated ascending fibres in the mamillary peduncle of the rabbit, chiefly to the lateral mamillary nucleus. Subsequent Marchi investigations by

Probst (1902), Bodian (1940), and Fox (1941) in several species have established endings of the mamillary peduncle in both the lateral and medial mamillary nuclei, especially the anteromedial part of the latter (see also Ramón y Cajal, 1955; Tello, 1936–37; Morin, 1950). Using the Nauta method in the rat, Guillery (1956) observed degeneration from the mamillary peduncle in the medial forebrain bundle and the diagonal band of Broca. Nauta & Kuypers (1958) in the cat have produced degeneration in the mamillary peduncle, medial forebrain bundle, and medial septal nucleus with lesions in the anteromedial pontine reticular formation. From the present study it is clear that the distribution of dorsal tegmental fibres in the mamillary peduncle coincides with the overall distribution of this tract. Degeneration in the medial, but not the lateral, mamillary nucleus was always accompanied by degeneration in the medial forebrain bundle. It is probable that some medial forebrain fibres are collaterals of axons supplying the medial mamillary nucleus, as observed by Tello (1936–37).

The mamillary nuclei receive hippocampal and pre-mamillary hypothalamic fibres via the fornix and medial forebrain bundle (Guillery, 1957) and send a prominent projection to the anterior thalamic nuclei, which in turn relay to the cingulate cortex (Cowan & Powell, 1954; Powell, 1958). Studies of the hippocampus and cingulate cortex by Kaada (1951) and MacLean (1957) suggest that these pathways may be concerned with autonomic and behavioural functions. Moreover, the mamillary bodies, in view of their important pre-mamillary and mesencephalic connexions, probably play a part in regulating descending impulses from the hypothalamus. Hence it is likely that the dorsal tegmental fibres in the mamillary peduncle are involved in central autonomic regulation by their projections to the mamillary, lateral hypothalamic, and medial septal nuclei, the last of which projects to the hippocampus (Daitz & Powell, 1954). Analysis of the role of the dorsal tegmental nucleus in this system should include the localized connexions between the dorsal tegmental and mamillary nuclear subdivisions and the fact that the latter can return impulses to the former by the mamillo-tegmental tract. The lateral mamillary nucleus may be the major source of such fibres (Guillery, 1956), which seem to have a localized ending in pars centralis of the dorsal tegmental nucleus (R. W. Guillery, unpublished).

(2) *Pons and midbrain*

In supplying fibres to the ventral tegmental area of Tsai and the posterior hypothalamic nucleus, the dorsal tegmental nucleus does not differ from more anterior regions of the central grey, but its connexions with nucleus medialis profundus, centralis superior, and the tegmental reticular nucleus may be established by collaterals of axons entering the mamillary peduncle. These nuclei are significant because of their hypothalamic (Sanz Ibañez, 1935; Guillery, 1957), limbic (Nauta, 1956, 1958), and cerebellar (Mettler & Zimmerman, 1943; Brodal, 1954) connexions. Furthermore, it is known that nucleus prepositus hypoglossi, with intense dorsal tegmental connexions, receives from (Thomas, Kaufman, Sprague & Chambers, 1956), and projects to (Brodal, 1952), the cerebellum. By these pathways the cerebellum may be involved in the central regulation of autonomic and behavioural functions (Chambers, 1947; Zanchetti & Zoccolini, 1954; Anand, Malhotra, Singh

& Dua, 1959). The significance of the dorsal tegmental connexions with these nuclei remains to be elucidated, but the present study reveals no direct dorsal tegmental connexions with the cerebellum nor is the author aware of any evidence for one.

(3) *Dorsal longitudinal fasciculus*

Schütz (1891) described in man the fasciculus which bears his name as a more or less diffuse collection of fine fibres situated in the central grey beside the central canal. In the upper cervical cord it surrounds the central canal as a corona. It passes between the dorsal motor vagal and hypoglossal nuclei and at pontine levels lies in the floor of the fourth ventricle near the midline (nucleus prepositus hypoglossi). In the posterior midbrain the fibres (corresponding to the ventral part of Ramón y Cajal's 'voie longitudinale périependymaire'; 1955, p. 193) collect ventral and lateral to the aqueduct but are dispersed laterally and dorsally at more anterior levels. Schütz did not describe the *dlf* proper beyond the mesencephalo-diencephalic junction, but he observed that tracts in the thalamic and hypothalamic periventricular regions are continuous with the *dlf*. As Ariëns Kappers, Huker & Crosby (1936, p. 1182) have remarked, the diencephalic periventricular system is part of the *dlf*; hence use of the term 'tractus periventricularis of Gurdjian' for this portion seems unnecessary. Schütz suggested that the *dlf* contains both ascending and descending fibres with connexions at all levels of the brain stem. Marburg (1931) and Ariëns Kappers *et al.* (1936) have reviewed the literature concerning the *dlf* in several species, whilst Thompson (1942) in the opossum and Crosby & Woodburne (1951) in the monkey have proposed many nuclear connexions besides those of Schütz. Of special interest is the great density of the fibre plexus observed in the dorsal tegmental nucleus, which is said to have reciprocal connexions with the *dlf*. Accordingly it has been proposed that the dorsal tegmental nucleus is 'a relay station for impulses passing between the diencephalic olfactory correlation centres and efferent centres of the brain stem' (Ariëns Kappers *et al.* 1936, p. 661).

Distribution. Few of the many proposed connexions of the *dlf* have been experimentally verified. Fibres have been shown to enter the central grey from the habenular nuclei (Bürgi & Bucher, 1955), the anterolateral spinal cord (Nauta & Kuypers, 1958), the bulbar reticular formation (Russell, 1954), and the cerebellum (Thomas *et al.* 1956). Fibres have been traced from the central grey to the inferior olive (Walberg, 1956), ventral tegmental area of Tsai, posterior hypothalamic nucleus and 'ventromediocaudal parts of massa intermedia' (Bucher & Bürgi, 1953), and widely to the mesencephalic tegmentum and tectum and hypothalamus (Nauta, 1958). In the present study degeneration in a rat with destruction of the mesencephalic *dlf* is recorded in detail. The intense hypothalamic projections indicate the importance in central autonomic activity of the *dlf*, which is already known to carry hypothalamic efferents (Guillery, 1957). Other axons from the central grey reach the hypothalamus after passing to the ventral tegmental area of Tsai and ascending to the posterior hypothalamic nucleus and medial forebrain bundle. The medial pre-mamillary hypothalamus, except the posterior periventricular nucleus and dorsal hypothalamic area, was notable for its lack of degeneration, although the impregnation revealed the very finest degenerated fibres in adjacent parts of the hypothalamus. Nevertheless, Nauta (1958) has briefly noted degeneration in

'medial hypothalamic cell groups' following destruction of the central grey of a cat. The bulbar *dlf* is distributed to nuclei prepositus hypoglossi and intercalatus and the upper cervical cord. A few fibres supply the dorsal motor vagal and hypoglossal nuclei. There are possibly connexions with the medial vestibular, motor glosso-pharyngeal, and superior salivatory nuclei, nucleus tractus solitarius, and the cervical ventral horn. No evidence was found for contributions to the motor trigeminal, abducens, or facial nuclei or to nucleus ambiguus. The descending system may be supplemented by secondary projections from nucleus prepositus hypoglossi to the nucleus of Roller and the medial reticular formation.

Dorsal tegmental nucleus. Since the *dlf* is a multisynaptic system, it is difficult to determine the ultimate destination of pathways in it from the dorsal tegmental nucleus alone with degeneration methods. However, it is clear that this nucleus must be a major, if not essential, synaptic station for fibres of the *dlf* both to and from the hypothalamus. Ascending fibres in the bulbar portion of the *dlf* synapse primarily in the posterior half of the nucleus, although many fibres or their collaterals also reach pars anterior. The anterior half of the dorsal tegmental nucleus gives rise to most of the ascending dorsal longitudinal axons from this nucleus. The bulk of the descending fibres in the mesencephalic *dlf* synapse throughout the dorsal tegmental nucleus, especially in pars anterior, whilst the *dlf* is continued by an intense bulbar projection of dorsal tegmental fibres.

Several investigations have pointed to close vagal-hypothalamic relationships (Ingram, 1940). It seems probable that the dorsal tegmental nucleus participates in such relationships. It is worth noting that, when stimulating the vagus nerve, Dell & Olson (1951*a*, *b*) recorded evoked potentials with the shortest latencies in regions to which the dorsal tegmental nucleus projects. Although there are reports that lesions in the ala cinerea produce 'retrograde changes' in the hypothalamic paraventricular nucleus of a dog (Urechia & Nitescu, 1925) and Marchi degeneration in the mamillary peduncle of opossums (Papez, 1932), sufficient details to permit evaluation of these claims are not available. The present evidence indicates that direct vago-hypothalamic fibres, if they exist, are not conveyed by the *dlf* or the mamillary peduncle in the rat. The dorsal longitudinal terminals in the dorsal motor vagal nucleus seem scarce, but this nucleus has an extremely poor fibre plexus (Ramón y Cajal, 1955, p. 247). It may be that nucleus intercalatus plays a key role in the mediation of impulses between the *dlf* and the dorsal vagal nuclei.

Although the functional characteristics of the dorsal tegmental nucleus are undetermined, several studies have implicated the dorsal isthmus region in bladder (Barrington, 1925; Tang, 1955) and respiratory (Johnson & Russell, 1952; Baxter & Olszewski, 1955) functions. Cragg (1959) produced a characteristic increase in the frequency and amplitude of respiration by stimulation at sites limited to the preoptic area, stria medullaris, habenular nuclei, interpeduncular nucleus, and dorsal tegmental nucleus, all of which are connected by a pathway involving the habenulo-peduncular tract and the pedunculo-tegmental tract of Ganser. Impulses conveyed in this pathway to the dorsal tegmental nucleus may reach bulbar respiratory neurons (Pitts, Magoun & Ranson, 1939) via nucleus prepositus hypoglossi and its projection to the medial medullary reticular formation. Other impulses may possibly descend through the *dlf* to motor cells of the phrenic nerve.

SUMMARY

1. Degeneration in the homolateral connexions of the dorsal tegmental nucleus was studied with the Nauta methods in rabbits and rats.

2. The nucleus is a major source of the fibres in the mamillary peduncle, which supply the mamillary nuclei, medial forebrain bundle, nucleus of the diagonal band of Broca, and medial septal nucleus.

3. Pars centralis projects to the anterior portion of the medial, and pars ventromedialis to the lateral mamillary nucleus.

4. Dorsal tegmental fibres also reach nuclei medialis profundus and centralis superior, the tegmental reticular nucleus, the ventral tegmental area of Tsai, and the posterior hypothalamic nucleus.

5. The nucleus is a major synaptic station for the pathways of the dorsal longitudinal fasciculus between the diencephalon and lower brain stem.

6. The dorsal tegmental nucleus and its connexions are probably involved in central autonomic regulation.

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LIST OF ABBREVIATIONS

A	Nucleus prepositus hypoglossi, pars gk 25
AC	Anterior commissure
AM	Anteromedial nucleus of the thalamus
BC	Brachium conjunctivum
BP	Brachium pontis
CM	Nucleus centrum medianum
Dark	Nucleus of Darkschewitsch
DBC	Decussation of brachium conjunctivum
DH	Dorsal hypothalamic area
DL	Dorsal nucleus of lateral lemniscus
DM	Dorsomedial nucleus of the thalamus
DTN	Dorsal tegmental nucleus
F	Fornix
Gra	Nucleus gracilis
I	Nucleus recessus incertus
Ic	Nucleus intercalatus
IP	Interpeduncular nucleus
LMN	Lateral mamillary nucleus
LR	Nucleus linea rostralis
LTN	Laterodorsal tegmental nucleus
MFB	Medial forebrain bundle
MH	Medial habenular nucleus
ML	Medial lemniscus
NCS	Nucleus centralis superior
NDB	Nucleus of the diagonal band
NMa	Nucleus medialis anterior
NMP	Nucleus medialis profundus
OC	Oculomotor complex
OT	Optic tract
P	Pyramidal tract
PC	Nucleus paracentralis of the thalamus

pc	Pars centralis of *DTN*
PF	Nucleus parafascicularis
PH	Posterior hypothalamic nucleus
pv	Pars ventromedialis of *DTN*
PrpH	Nucleus prepositus hypoglossi
PT	Pretectal nucleus
R	Nucleus of Roller
RN	Red nucleus
RPO	Pontine reticular nucleus, pars oralis
SMe	Stria medullaris
TD	Tegmental decussations
TRN	Tegmental reticular nucleus
VL	Ventral nucleus of lateral lemniscus
VM	Ventromedial nucleus of the thalamus
III	Oculomotor nucleus
MLF	Medial longitudinal fasciculus
MMN	Medial mamillary nucleus
MP	Mamillary peduncle
MR	Medial medullary reticular formation
NA	Nucleus accumbens
IV	Trochlear nucleus
V	Mesencephalic trigeminal nucleus
*G*VII	Genu of the facial nerve
X	Dorsal motor vagal nucleus
XII	Hypoglossal nucleus

EXPLANATION OF PLATES

All sections are in the parasagittal plane and, except in Fig. 1, have been prepared by the Nauta–Gygax method. All degeneration shown is ipsilateral to the lesions.

Plate 1

Fig. 1. Dorsal tegmental nucleus of a rat. Frozen section, cresyl violet. ×45.

Fig. 2. Massive degeneration in the mamillary peduncle of rabbit DT1 following a dorsal tegmental lesion. Contrast the normal oculomotor (vertical) fibres, some of which are incompletely suppressed but none of which meet any of the author's criteria for degeneration (see Materials and Methods). ×150.

Fig. 3. From the contralateral mamillary peduncle of rabbit DT1. The vertical oculomotor fibres and large horizontal fibres of the mamillary peduncle are unquestionably normal. This particular section was chosen to show the finer fibres of the mamillary peduncle which, due to a low degree of suppression, are stained intermittently at regular intervals but are not broken up and are seen in specimens without lesions. ×160.

Plate 2

Fig. 4. Massive degeneration in the lateral mamillary nucleus of rabbit DT1 following a dorsal tegmental lesion. ×100.

Fig. 5. From previous field. ×400.

Fig. 6. Dense degeneration in the medial mamillary nucleus of rabbit DT3 following a dorsal tegmental lesion. Note the normal efferent (vertical) fibres, to the right (posterior) of which there is no degeneration. ×130.

Fig. 7. Degeneration from previous field. ×400.

Fig. 8. Typical normal fibres from the contralateral lateral mamillary nucleus of rabbit DT1 (field corresponding to Fig. 5). ×400.

Plate 3

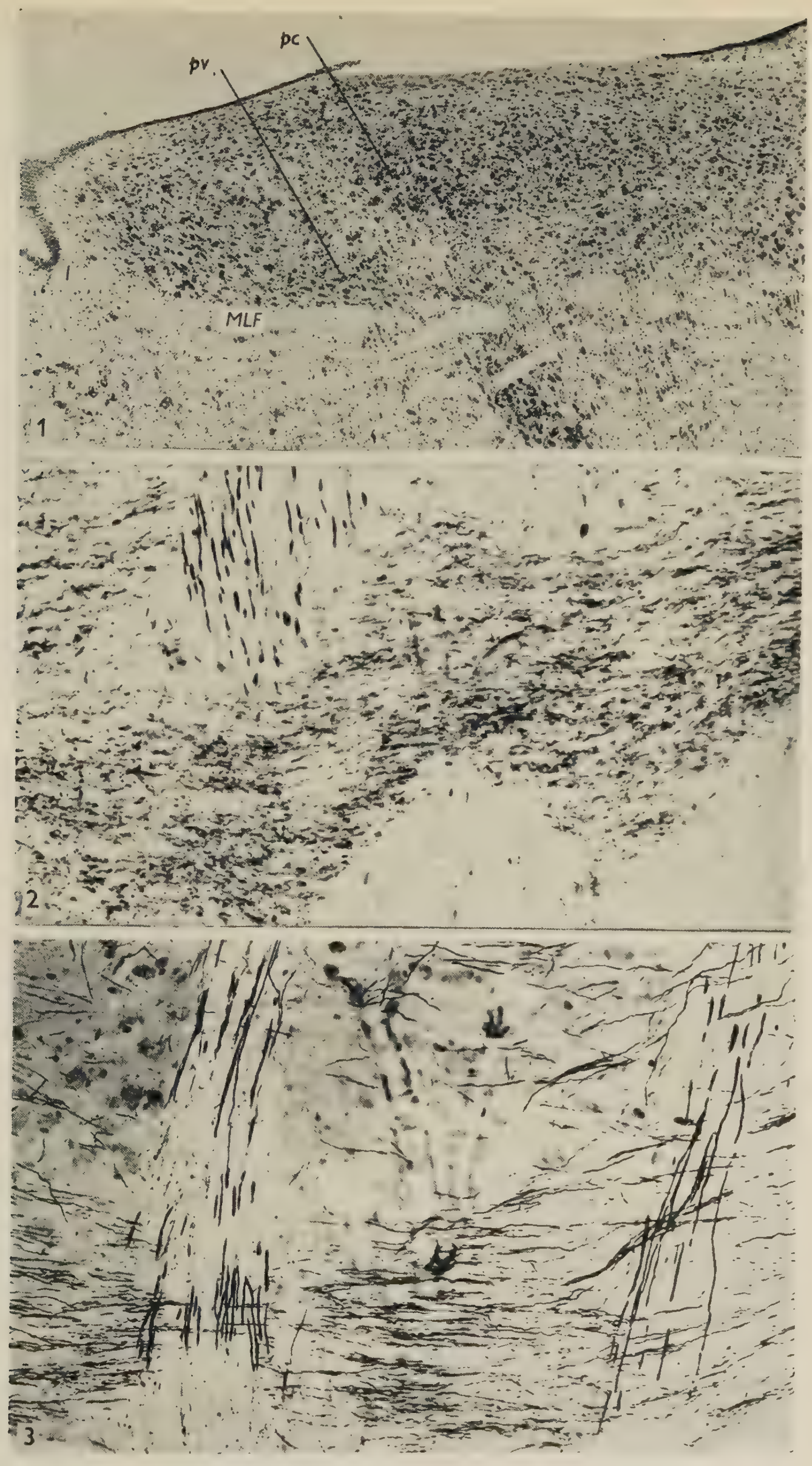
Fig. 9. Moderate degeneration in the dorsal longitudinal fasciculus of rabbit DT1 following a dorsal tegmental lesion. ×480.

Fig. 10. Normal fibres and nuclei of cells from the dorsal longitudinal fasciculus of an unoperated rabbit in a section simultaneously impregnated with that of Fig. 9. ×480.

Fig. 11. Massive degeneration of the dorsal longitudinal fasciculus at the mesencephalo-diencephalic junction following destruction of the central grey of rat 474. ×120.

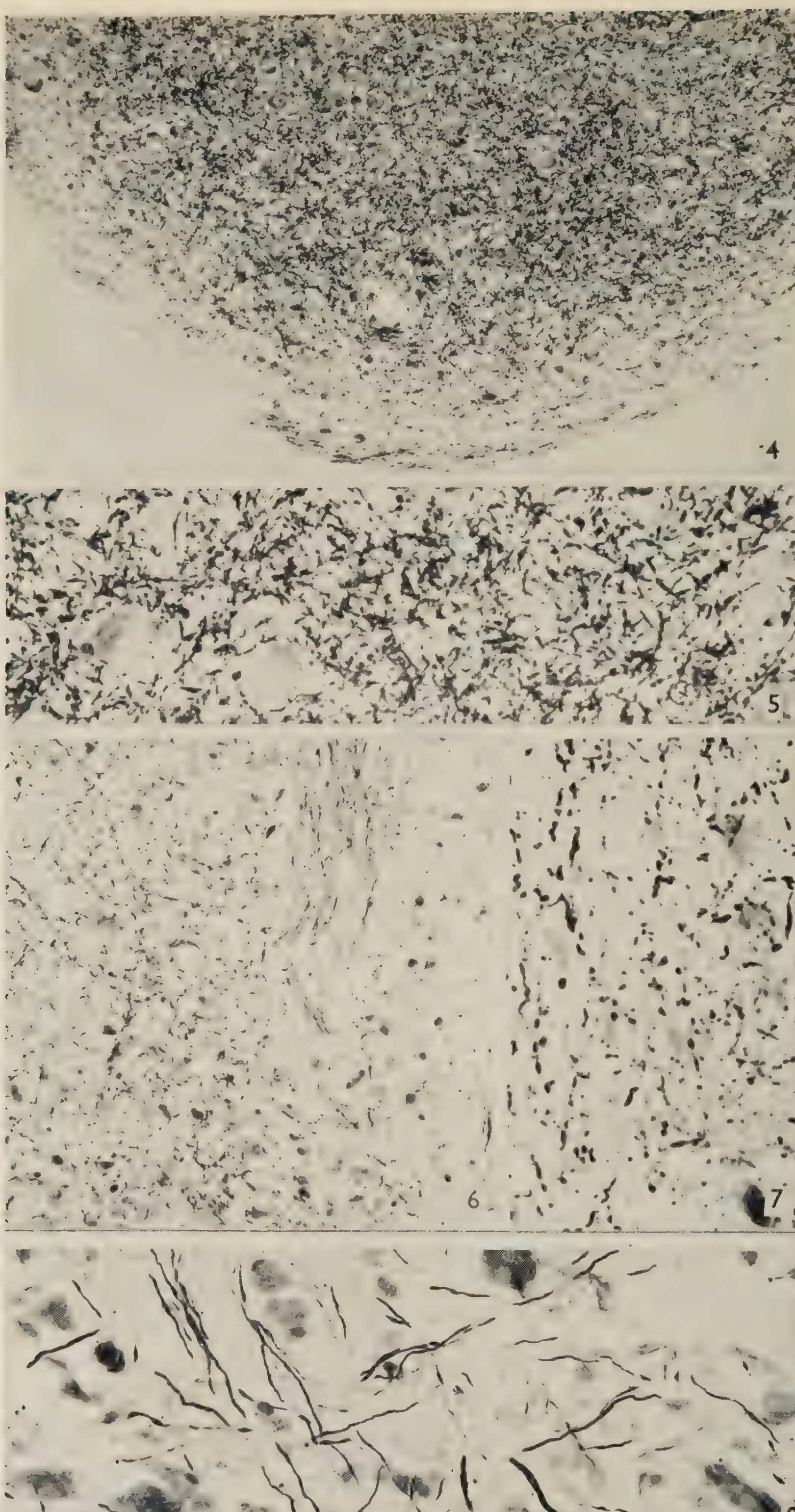
Fig. 12. Degeneration in nucleus prepositus hypoglossi of rabbit T8 following a dorsal tegmental lesion. Fibres of passage concentrate dorsally (upper part of field) whilst ventrally fibres are dispersed among the cell bodies. ×320.

Fig. 13. Degeneration in pars posterior of the dorsal tegmental nucleus of rat 481 following a lesion in nucleus prepositus hypoglossi. Note the pericellular coiling that characterizes preterminal degeneration. ×320.

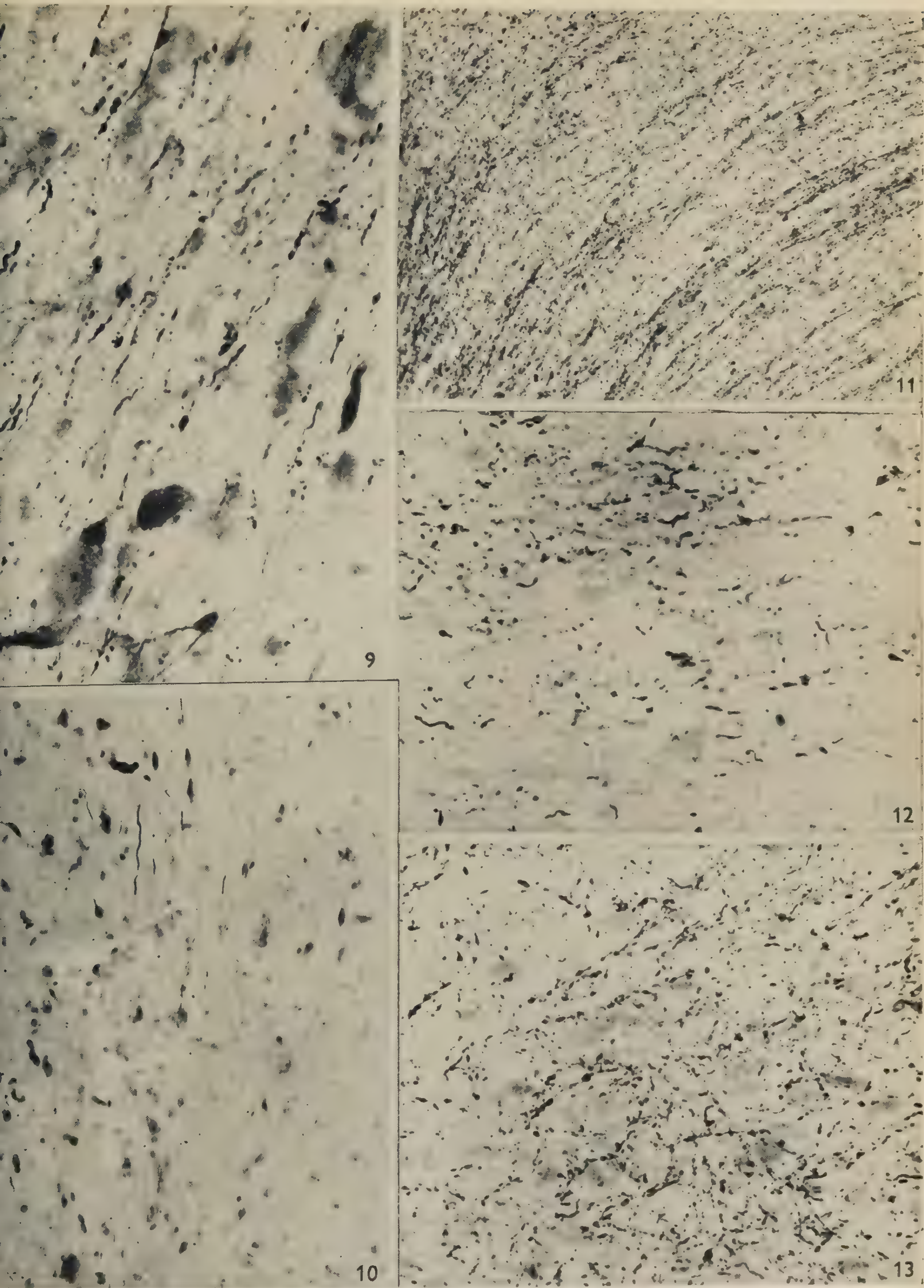


MOREST—Dorsal tegmental connexions

(*Facing p.* 246)



MOREST—DORSAL TEGMENTAL CONNEXIONS



MOREST—DORSAL TEGMENTAL CONNEXIONS

GRANULATION TISSUE RESORPTION DURING FREE AND LIMITED CONTRACTION OF SKIN WOUNDS

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INTRODUCTION

Large excised skin wounds in animals, in areas where the skin is loose, undergo contraction in the early stages of repair (e.g. Billingham & Reynolds, 1952). The term 'contraction' refers to the process whereby the intact skin bordering the deficit is drawn inwards, reducing the area of the wound. That this process occurs in man is suggested by the observations of Bailey (1945).

Contraction of small wounds in rats is accompanied by loss of substance from the granulation tissue within the wound and a progressive rise of collagen both in concentration and in absolute amount (Abercrombie, Flint & James, 1954). Grillo, Watts & Gross (1958) found loss of substance including collagen during the later phase of contraction in guinea-pig wounds. They concluded that the total amount of collagen increased rapidly between the 5th and 8th days, and diminished rapidly between the 8th and 10th days. Abercrombie, James & Newcombe (1960) working with rabbit wounds whose contraction was hindered by a splinting technique, noted that contraction occurring rapidly after removal of the splints was associated with absolute loss of material, including collagen, but not apparently with changes in granulation tissue composition per unit wet weight. These observations, however, cannot be regarded as entirely satisfactory, since they were not based upon the analysis of strictly comparable granulation tissue areas (see Discussion).

The present experiments were designed to overcome this difficulty, and to elucidate the effects of wound contraction upon the composition and amount of granulation tissue within areas defined before contraction began.

Tattoos were used to mark an equal area of granulation tissue in each of a series of skin wounds in rabbits. This tattooing was performed on the 10th post-operative day, and until that time contraction was in all cases impeded by splints applied to the wound margins immediately after operation. If splints were removed when granulation tissue tattooing was completed on the 10th day, extensive wound contraction had occurred by the 12th. This contraction was accompanied by marked diminution of the tattooed granulation tissue areas. We have compared the composition of such contracted, tattooed, areas with that of equivalent areas from uncontracted, splinted, wounds. Composition was determined in terms of wet weight, water, collagen, and non-collagenous nitrogen.

The term 'equivalent area' simply implies equality of the tattooed granulation tissue areas at the time of their original definition on the 10th day. Clearly they later differed considerably in contracted and uncontracted wounds.

MATERIAL AND METHODS

Adult male rabbits (mean weight 2·3 kg., range 1·7–3·3, S.E. 0·15) were used. They were anaesthetized with intravenous Nembutal, and hair was removed from each side of the thorax with electric clippers. Animals showing either heavy pigmentation or active hair growth in the proposed operation area were rejected at this stage, since these features interfered with the tattooing and splinting techniques employed.

Animals (twenty in all) satisfactory in these respects were given a further dose of Nembutal (0·2 g./kg.) intraperitoneally, and tied with their forelimbs in extension. The techniques employed have been fully described elsewhere (Abercrombie *et al.* 1960), but briefly the procedure was as follows. A 2×2 cm. square was marked on the skin by sixteen symmetrically arranged tattooed dots, using Indian ink and a no. 22 straight cutting needle held in a small engineer's chuck. The skin within the square was aseptically excised down to the panniculus, and a Perspex splint, with a central aperture corresponding to the wound, glued to the skin around it immediately afterwards. The tattoos were traced on to cellophane before and after operation, and after the splint was affixed. The wound was dressed with tulle gras and sterile gauze, and the whole procedure repeated on the contralateral side. Finally, a light plaster cast was applied and 200,000 units of penicillin were given intramuscularly. Skin excised at operation was weighed in stoppered bottles.

At 10 days, again under anaesthesia, dressings were removed. A 1×1 cm. square was then tattooed, using eight tattoo marks, on the central part of the granulation tissue within the wound, and a further tracing taken. There were three subsequent treatment groups.

In the first, the area enclosed by the granulation tissue tattoo was immediately excised for analysis, the splint remaining undisturbed.

In the second, wound and splint were left undisturbed after tattooing and tracing on the 10th day, and redressed as before. On the 12th day after initial operation (again under anaesthesia) a tracing was taken and the tattooed granulation tissue area excised, still with the splint in position.

In the third the splint was removed after tattooing and tracing on the 10th day. Twenty minutes later the tattoos were traced again, and the animal redressed. On the 12th day, a further tracing was made and the tattooed granulation tissue area excised.

All excisions were made leaving the panniculus intact in the wound bed.

From the cellophane tracings further tracings were made on to paper of standard thickness. These were then cut out and weighed, and results calculated as cm.^{2}.

CHEMICAL METHODS

Each specimen was dried to constant weight at 102° C. Collagen was then brought into solution with hot trichloroacetic acid (TCA) (Fitch, Harkness & Harkness, 1955). The tissue was first treated with 5 ml. of 5% TCA at 90° C. for 30 min. It was then homogenized in a Potter–Elvehjem type homogenizer, made to twice the original volume with 10% TCA, and heated at 90° C. for a further 30 min. Volume was corrected if necessary with 10% TCA, and two 1·0 ml. volumes were removed for estimation of nitrogen by a micro-Kjeldahl method (Ma & Zuazaga, 1942). The

remaining homogenate was centrifuged at 2500 r.p.m. for 10 min., and two 1·0 ml. volumes of the supernatant removed. 1·0 ml. of 12N-HCl was added to each, and the tubes sealed and autoclaved at 2·8 kg./cm.^{2} pressure for 4 hr. Hydroxyproline in the hydrolysate was then estimated by the method of Neuman & Logan (1950) as modified by Martin & Axelrod (1953).

Hydroxyproline values were multiplied by a factor of 7·46 to provide estimates of collagen. Non-collagen nitrogen (NCN) was estimated by subtracting collagen from total nitrogen.

RESULTS

The total population of forty wounds had a mean skin tattoo area before operation of $3·85 \pm 0·05$ cm.^{2}. After excision of the enclosed skin area (wet weight $311·3 \pm 16·0$ mg.) the wound margins retracted and tattoo area increased to $6·06 \pm 0·08$ cm.^{2}. Splinting diminished tattoo area to $5·92 \pm 0·11$ cm.^{2}. By the 10th day skin tattoo area had significantly ($P < 0·001$) diminished to $5·20 \pm 0·10$ cm.^{2}, demonstrating that splinting had not completely suspended wound contraction. At this time granulation tissue tattoos were made, enclosing a mean area of $0·93 \pm 0·01$ cm.^{2}.

The results of subsequent weighing and analysis of excised granulation tissue areas may be presented in two ways: first, to show changes in the absolute amounts of material present in equivalent (although not equal) areas when, beyond the 10th day, contraction is either hindered or allowed freely to proceed; secondly, to show changes in concentration of the granulation tissue components measured, i.e. in terms of unit area or unit wet weight.

All tests of significance in 'Results' are derived from estimates of the extent to which mean side to side differences for a given parameter depart significantly from zero in the group of animals concerned.

Absolute changes in composition

In seven animals the areas delimited by the granulation tissue tattoo were excised immediately after tattooing, and without disturbing the splints, on the 10th day (treatment A). On the contralateral side the splint was left undisturbed, but biopsy was postponed until the 12th day (treatment B).

On the 10th day mean skin and granulation tissue tattoos enclosed areas of $5·06 \pm 0·22$ cm.^{2} and $0·94 \pm 0·03$ cm.^{2}, respectively, in treatment A wounds before biopsy. In treatment B wounds on the 10th day these areas were $5·50 \pm 0·22$ and $0·94 \pm 0·03$ cm.^{2}. In neither skin nor granulation tissue tattoos did the mean of the differences between sides significantly exceed zero, and the wounds constituting the treatment groups may therefore be considered comparable.

Despite continued splinting between the 10th and 12th days treatment B skin tattoo areas were reduced by $0·58 \pm 0·17$ cm.^{2}, and granulation tissue tattoos by $0·27 \pm 0·08$ cm.^{2}. Mean decreases were in both cases significant ($0·02 > P > 0·01$). Thus an area of $0·94 \pm 0·03$ cm.^{2} on the 10th day was reduced in treatment B wounds to $0·67 \pm 0·03$ cm.^{2} at biopsy on the 12th day.

The results of analysis of the excised granulation tissue are shown in Table 1. Despite the area change already noted, neither wet weight, water content, collagen, nor NCN differ in absolute amount between wounds accorded treatment A and those

accorded treatment B. In this case, then, reduction in area was unaccompanied by absolute loss of substance.

A further group of seven animals again received treatment A on one side. Splints were, however, removed from the contralateral wounds after the granulation tissue was tattooed on the 10th day (treatment C). Before this removal the two sets of wounds were again not significantly different in area. Those receiving treatment A had skin and granulation tissue tattoos of mean area $4{\cdot}94 \pm 0{\cdot}31$ and $0{\cdot}91 \pm 0{\cdot}05$ cm.^{2}, those receiving treatment C $4{\cdot}98 \pm 0{\cdot}25$ and $0{\cdot}89 \pm 0{\cdot}03$ cm.^{2}.

Splint removal in treatment C wounds was followed by immediate and significant contraction of both skin and granulation tissue tattoos. The former lost $1{\cdot}39 \pm 0{\cdot}16$ cm.^{2} and the latter $0{\cdot}21 \pm 0{\cdot}03$ cm.^{2} within 20 min. ($P < 0{\cdot}001$ in each case). Further contraction had occurred by the 12th day, when mean skin tattoo area was reduced to $2{\cdot}55 \pm 0{\cdot}21$ and the granulation tissue tattoo area to $0{\cdot}39 \pm 0{\cdot}04$ cm.^{2}.

Analysis of the excised granulation tissue is shown in Table 1. In this case free contraction between 10 and 12 days was accompanied by significant reduction of each parameter. Wet weight was reduced by $56{\cdot}2 \pm 8{\cdot}2$ mg., water by $47{\cdot}6 \pm 6{\cdot}7$ mg. collagen by $2{\cdot}48 \pm 0{\cdot}27$ mg. (in each case $P < 0{\cdot}001$), and NCN by $0{\cdot}58 \pm 0{\cdot}21$ mg. ($0{\cdot}05 > P > 0{\cdot}02$).

Table 1. *Total amounts of material in equivalent areas*

Treatment	Wet wt.	Water	Collagen	NCN
A	$127{\cdot}8 \pm 11{\cdot}2$	$109{\cdot}5 \pm 10{\cdot}3$	$4{\cdot}30 \pm 0{\cdot}32$	$2{\cdot}06 \pm 0{\cdot}18$
B	$118{\cdot}5 \pm 7{\cdot}8$	$103{\cdot}8 \pm 6{\cdot}9$	$4{\cdot}34 \pm 0{\cdot}35$	$1{\cdot}94 \pm 0{\cdot}14$
(7 animals)				
A	$152{\cdot}9 \pm 13{\cdot}9$	$131{\cdot}1 \pm 11{\cdot}6$	$6{\cdot}23 \pm 0{\cdot}67$	$2{\cdot}25 \pm 0{\cdot}20$
C	$96{\cdot}7 \pm 12{\cdot}6$	$83{\cdot}5 \pm 11{\cdot}3$	$3{\cdot}75 \pm 0{\cdot}52$	$1{\cdot}67 \pm 0{\cdot}25$
(7 animals)				
B	$143{\cdot}9 \pm 13{\cdot}0$	$129{\cdot}1 \pm 11{\cdot}3$	$4{\cdot}93 \pm 0{\cdot}51$	$2{\cdot}04 \pm 0{\cdot}24$
C	$93{\cdot}5 \pm 13{\cdot}1$	$87{\cdot}8 \pm 11{\cdot}4$*	$3{\cdot}39 \pm 0{\cdot}58$	$1{\cdot}48 \pm 0{\cdot}22$
(6 animals)				

Treatment A. Splinted wound biopsied on the 10th day.
Treatment B. Splinted wound biopsied on the 12th day.
Treatment C. Splint removed on the 10th day, wound biopsied on the 12th.
* Five animals only.

The last absolute comparison to be made is between sides of six animals in which both wounds were biopsied on the 12th day. On one side (treatment B) splints were retained until biopsy. On the other (treatment C) they were removed on the 10th day. Skin tattoos of treatment B wounds ($5{\cdot}60 \pm 0{\cdot}02$ cm.^{2}) slightly exceeded those of the contralateral sides ($5{\cdot}18 \pm 0{\cdot}26$ cm.^{2}) in area ($0{\cdot}05 > P > 0{\cdot}02$). Granulation tissue areas, however, were not significantly different ($0{\cdot}97 \pm 0{\cdot}04$ cm.^{2} for B and $0{\cdot}93 \pm 0{\cdot}03$ cm.^{2} for C).

Removal of splints from treatment C wounds produced area changes similar to those described above for animals receiving A and C treatments. Immediate contraction followed splint removal, and by the 12th day skin and granulation tissue tattoos were further reduced to $2{\cdot}45 \pm 0{\cdot}25$ and $0{\cdot}37 \pm 0{\cdot}03$ cm.^{2}. Continued splinting again failed totally to suppress contraction, since by the 12th day, in treatment B wounds, skin and granulation tissue tattoo areas were diminished to $4{\cdot}98 \pm 0{\cdot}27$ and $0{\cdot}69 \pm 0{\cdot}04$ cm.^{2}.

Table 1 shows the results of analysis of the excised material. In respect of all indices measured, granulation tissue from splinted wounds exceeded that from wounds allowed to contract between the 10th and 12th days. Mean wet weight difference was $50{\cdot}4 \pm 11{\cdot}1$ mg. ($0{\cdot}01 > P > 0{\cdot}001$), collagen difference $1{\cdot}54 \pm 0{\cdot}45$ mg. ($0{\cdot}02 > P > 0{\cdot}01$), and NCN difference $0{\cdot}56 \pm 0{\cdot}19$ mg. ($0{\cdot}05 > P > 0{\cdot}02$). In this group one dry-weight determination was not recorded. A mean difference in water content of $39{\cdot}0 \pm 7{\cdot}4$ mg. was found for the five remaining animals ($0{\cdot}02 > P > 0{\cdot}01$).

Analysis of granulation tissue in terms of unit area and weight

Results are shown in Tables 2 (unit area) and 3 (mg./g. wet weight). It has already been established that in the first group of animals, receiving A and B treatments, some contraction occurred in the latter despite splinting until the 12th day, but that this contraction was not accompanied by absolute loss of granulation tissue substance. As would be expected, therefore, wet weight, water, collagen, and NCN

Table 2. *Concentrations per unit area* (*mg./cm*^{2})

Treatment	Wet wt.	Water	Collagen	NCN
A	135·4 ± 11·2	115·9 ± 10·4	4·57 ± 0·36	2·19 ± 0·19
B	177·5 ± 9·7	155·5 ± 11·9	6·51 ± 0·56	2·90 ± 0·22
(7 animals)				
A	168·1 ± 13·8	144·3 ± 11·7	6·81 ± 0·61	2·47 ± 0·20
C	251·9 ± 24·0	217·0 ± 21·9	9·67 ± 1·44	4·34 ± 0·57
(7 animals)				
B	207·1 ± 11·2	161·4 ± 3·0	7·07 ± 0·45	2·93 ± 0·27
C	245·9 ± 17·4	227·3 ± 14·8	8·89 ± 0·89	3·91 ± 0·30
(6 animals)				

Treatment A. Splinted wound biopsied on the 10th day.
Treatment B. Splinted wound biopsied on the 12th day.
Treatment C. Splint removed on the 10th day, wound biopsied on the 12th.

Table 3. *Concentrations per unit wet weight* (*mg./g.*)

Treatment	Water	Collagen	NCN
A	854·3 ± 10·9	34·40 ± 2·47	16·16 ± 0·62
B	876·1 ± 10·2	36·54 ± 1·59	16·49 ± 0·74
(7 animals)			
A	858·4 ± 5·1	40·46 ± 1·27	14·80 ± 0·57
C	858·4 ± 9·4	38·93 ± 2·90	17·32 ± 1·56
(7 animals)			
B	898·6 ± 7·5	34·08 ± 0·89	14·14 ± 0·92
C	893·7 ± 12·1	36·38 ± 4·81	16·03 ± 0·89
(6 animals)			

Treatment A. Splinted wound biopsied on the 10th day.
Treatment B. Splinted wound biopsied on the 12th day.
Treatment C. Splint removed on the 10th day, wound biopsied on the 12th.

per unit area all increased significantly in the 2 days between the 10th and 12th after operation when comparison was made with equivalent 10-day granulation tissue areas (for each $0{\cdot}01 > P > 0{\cdot}001$). This increase in mass per unit area, almost certainly reflecting an increase in thickness, was accompanied by no significant change in either water, collagen, or NCN per unit weight.

A and C treatments showed that free contraction from the 10th to the 12th day was also accompanied by significant increases in wet weight ($P < 0{\cdot}001$), water ($P < 0{\cdot}001$), collagen ($0{\cdot}05 > P > 0{\cdot}02$), and NCN ($0{\cdot}01 > P > 0{\cdot}001$) per unit area. Expressed in terms of wet weight, water, collagen, and NCN underwent no significant change.

This similarity of the granulation tissue in composition in terms of wet weight, and dissimilarity in terms of unit area, is shown also in the last group of animals (B–C treatments). 12-day tissue from unsplinted wounds weighed more per unit area ($0{\cdot}02 > P > 0{\cdot}01$), had more water ($0{\cdot}02 > P > 0{\cdot}01$), and more NCN ($0{\cdot}01 > P > 0{\cdot}001$) than tissue from splinted wounds of the same age. There was, however, no significant difference in collagen content. Water, collagen, and NCN indices were similar when expressed in terms of wet weight.

DISCUSSION

When comparison is to be made between the absolute amounts of material present within contracted and uncontracted wounds it is clearly necessary that the areas excised from each should be strictly comparable. Methods involving the excision of areas of granulation tissue defined only at the time of biopsy are unsatisfactory, since the assumption is made that granulation tissue contracts uniformly, and is of homogeneous composition.

On the other hand, excision of the whole wound content unadulterated by the surrounding skin margin presents considerable technical difficulties.

For these reasons we have based our analyses upon standard areas of granulation tissue demarcated within all wounds on the 10th day after operation, and until this time contraction was in all cases largely prevented by a splinting technique. Subsequent splint removal enable the changes consequent upon the rapid contraction that followed (Abercrombie *et al.* 1960) to be studied.

Our results make it clear that free contraction between 10 and 12 days is associated with an absolute loss of wet weight, water, collagen, and NCN when comparison is made with equivalent granulation tissue areas at 10 days (A–C treatments). That this loss is not merely a consequence of ageing of the tissue is indicated by the group of animals receiving treatments B and C. In them, the diminished (but still significant) contraction in 'B' wounds by the 12th day resulted in significantly greater retention of the tissue components measured than was found in the contralateral, freely contracting, 'C' wounds. Again, areas from wounds splinted to the 12th day contained absolute amounts of material no different from equivalent areas on the 10th day (treatments A and B). It appears, therefore, that whereas a loss in area of about 30% between the 10th and 12th days was unaccompanied by detectable absolute loss of material (A–B treatments), a more acute contraction of some 60% (A–C treatments) resulted in the absolute reduction of each granulation tissue component measured.

Resorption of granulation tissue during contraction does not occur symmetrically in the vertical and horizontal dimensions, for while area is diminished, thickness is increased. This is shown when results of analysis are expressed in terms of unit area (Table 2). A consistent increase occurred from the 10th to the 12th day, both

in wounds whose contraction was unimpeded during this period (A–C treatments) and in those whose contraction was hindered, but not suppressed, by continued splinting (A–B treatments). Also, with the single exception of collagen, freely contracted wounds had higher unit area indices than those of equal age whose contraction was impeded (B–C treatments).

Finally, resorption and increase in thickness were not accompanied by changes in concentration (Table 3). Results in terms of unit wet weight show that water, collagen, and NCN remained remarkably constant throughout.

Our results, then, show that in wounds whose contraction is first impeded by splinting until the 10th post-operative day, and then (by removal of the splints) is allowed freely to occur until the 12th post-operative day, there is an absolute loss of material from granulation tissue areas defined on the 10th day. During contraction, such areas get thicker and maintain an apparently stable composition. When the extent of contraction is reduced between the 10th and 12th days by continued splinting, however, no detectable loss of material occurs.

The mechanism whereby the centripetal movement of the wound edges is achieved is of obvious relevance to the interpretation of these results. First, we have criticized elsewhere (Abercrombie *et al.* 1960) the view that contraction is simply a consequence of the withdrawal of material from the wound, as proposed by Cuthbertson (1959). It is difficult to envisage a mechanism for such withdrawal that could result in contraction in one plane (the horizontal, to draw the skin edges together) and expansion in another at right angles to the first (to explain the increase in thickness). Loss of granulation tissue substance, then, is more likely to be a phenomenon associated with contraction than to be its cause.

There is little doubt, however, that wound contraction occurs as a consequence of contraction of the granulation tissue within the wound (Abercrombie *et al.* 1960). Further, this contraction is independent of newly formed collagen, and is probably produced, in a manner not yet understood, by the cell population within the repair area (Abercrombie, Flint & James, 1956).

Seen in this perspective, our present results suggest that if the cells within a wound are prevented from imposing a rapid shape change (contraction) upon the tissue mass of which they are part, then they fail to mediate resorption. If on the other hand rapid shape change is allowed, such resorption occurs.

An obvious possibility is that the amount of material within a wound is determined by the balance between simultaneous processes of deposition and resorption. Hass (1940), working with fibroblast cultures implanted in granulation tissue, but protected by semipermeable membranes, postulated that fluid exudates from granulation tissue might exert two antagonistic influences of this kind on collagen. Our findings suggest that the extent of the resorptive phase may be influenced by the rapidity and extent of wound contraction.

That tissue resorption is not unique to the rapid contraction following desplinting is shown by a number of reported analyses of the content of wounds contracting without impairment. Absolute loss of wet weight has been observed, as examples, in large (2 cm.^{2}) wounds of guinea-pig skin and in small (25 mm.^{2}) wounds of rat skin (Abercrombie *et al.* 1954). Reduction of total collagen content during contraction was reported by Grillo *et al.* (1950) in the guinea-pig between the 8th

and 10th post-operative day. While Abercrombie *et al.* (1954) failed to detect collagen resorption in rat wounds, their biopsies were made on the 5th and 10th post-operative days. It remains possible, as Grillo *et al.* (1958) suggested, that a peak of collagen production, followed rapidly by resorption, occurred between these times. Abercrombie & James (unpublished), however, found no support for this hypothesis from analysis of the content of 25 mm.^{2} wounds in the rat at 5, 7, and 10 days. Thus it may be that the absolute extent of contraction determines whether or not collagen resorption occurs, and in this respect small wounds may behave differently from large ones.

Increase in collagen concentration per unit wet weight during contraction has been reported by Grillo *et al.* (1958) in guinea-pig wounds and in rat wounds by Abercrombie *et al.* (1954); Dunphy & Udupa (1955) found an increase in collagen concentration in incised wounds of rats from the 3rd to the 12th day, a period embracing that in which wound contraction occurs. Our apparently anomalous present finding, that 'acute' contraction is unaccompanied by an increase in collagen concentration, has a number of possible explanations. Collagen concentration, like total collagen content, may be influenced by the rate and extent of contraction. Alternatively, the time interval between our analyses (2 days) may have been too short to enable an increase in concentration to have become sufficiently marked to be detected.

Collagen is known to be metabolically relatively inert compared to other body proteins (Neuberger, Perrone & Slack, 1951; Neuberger & Slack, 1953) but two special features may bear on its over-all resorption from granulation tissue within large contracting skin wounds. First, it is known that loss of collagen under conditions of severe protein deficiency occurs more readily from skin than from other tissues (Harkness, Harkness & James, 1958). Thus skin collagen may possess greater lability than that elsewhere in the body. Secondly, it is likely that the solubility and maturity of collagen are inversely related. Its diminished extractability with increasing age of the animal (Banfield, 1952) may be associated with more stable association of its molecules to form cross-striated fibrils (Gross, 1958), and immature fibrils of the kind seen in foetal development have been observed in granulation tissue (Banfield, 1955).

SUMMARY

1. In rabbits areas about 1×1 cm.^{2} were tattooed on the granulation tissue within wounds whose contraction had been impeded to the 10th post-operative day by a splinting technique.

2. Such areas were excised, weighed, and analysed for water, collagen, and non-collagenous nitrogen (NCN), on the 10th day, on the 12th day with contraction still impeded by splinting, and on the 12th day when splints had been removed on the 10th.

3. The free contraction between 10 and 12 days consequent upon splint removal on the 10th resulted in a diminution of the tattooed area by some 60 %, associated with absolute loss of wet weight, water, collagen, and NCN. This loss was prevented when splinting was continued to the 12th day, although some contraction (30 % area diminution) still occurred.

4. A marked change in shape (free contraction) thus leads to granulation tissue resorption, while a less-marked change (impeded contraction) does not.

5. It appears that mechanisms of resorption and deposition may determine the amount of granulation tissue within wounds, the former predominating when rapid and extensive change in shape occurs.

6. While wet weight, water, collagen, and NCN increased in terms of unit area in free contraction, water, collagen, and NCN were unchanged in terms of unit wet weight.

7. The absolute loss of collagen observed is of interest in view of its known metabolic inertia.

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PIGMENTATION IN THE NUCLEUS SUBSTANTIAE NIGRAE OF MAMMALS

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INTRODUCTION

The presence of black or brown intracytoplasmic granules, usually considered to be melanin, in the cells of certain nuclei of the human brain stem has long been recognized. This pigment is concentrated chiefly in the nucleus substantiae nigrae of the midbrain and the nucleus locus coeruleus of the pons. Although many earlier writers assumed that pigment granules are present only in man, such pigmentation is present in other mammals; in the gorilla (Adler, 1942), the chimpanzee, orang utan, gibbon, certain Old World monkeys and lemurs (Scherer, 1939), in the cat and dog (Brown, 1943) and in the horse (Gillilan, 1943). There have been no reports of pigmentation in the nucleus substantiae nigrae of other mammals. Therefore, it was decided to examine the brains of as wide a series of mammals as possible, in order to determine which of these animals contain pigment granules in the nucleus substantiae nigrae, in the hope that the distribution of pigmentation within the Mammalia might give some indication of its function in the brain.

MATERIALS AND METHODS

The following mammals have been studied:

Primates: man (*Homo sapiens*), chimpanzee (*Pan satyrus*), gibbon (*Hylobates mulleri*), white-collared mangeby (*Cerocebus torquatus*), patas monkey (*Cercopithecus patas*), macaque monkey (*Macaca rhesus*), black and white colobus monkey (*Colobus* species), baboon (*Papio* species), weeper capuchin monkey (*Cebus capucinus*), cebus monkey (*Cebus* species), Humboldt's woolly monkey (*Lagothrix humboldtii*), lion marmoset (*Lentocebus rosalia*), black-tailed marmoset (*Callithrix argentata*), black-fronted lemur (*Lemur nigrifrons*), brown lemur (*Lemur fulvus*), Malayan tree shrew (*Tupaia belangeri*).

Lagomorpha: rabbit (*Oryctolagus curiculus*).

Rodentia: grey squirrel (*Sciurus carolinensis*), mouse (*Mus murus*), rat (*Rattus norwegicus*), guinea-pig (*Cavia cabensis*).

Carnivora: dog (*Canis canis*), fox (*Vulpes vulpes*), European badger (*Meles meles*), sand-pig badger (*Arctonyx collaris*), Canadian skunk (*Mephitis mephitis*), otter (*Lutra lutra*), Indian civet (*Viverra malaccensis*), African genet (*Genetta pardina*), domestic cat (*Felis felis*), pampas cat (*Felis dendrailurus*), seal (*Phoca phoca*).

Perissodactyla: horse (*Equus equus*).

Artiodactyla: pig (*Sus scrofa*), yellow-backed duiker (*Cephalophus sylvicultrix*), sheep (*Ovis aries*), musk deer (*Moschus moschiforus*), hog deer (*Cervus porcinus*).

Chiroptera: Indian fruit bat (*Pteropus giganteus*).

Insectivora: hedgehog (*Erinaceus* species).

Xenarthra: six-banded armadillo (*Euphractus sexcinctus*).

Marsupialia: rufous rat kangaroo (*Aepyprymnus rufescens*), tree kangaroo (*Dendrolagus* species), fat-tailed sminthopsis (*Sminthopsis crassicaudata*), Tasmanian barred-bandicoot (*Perameles gunni*), southern short-nosed bandicoot (*Isoodon obesulus*), common ring-tail (*Pseudocheirus laniginosus*), Tasmanian ring-tail (*Pseudocheirus convolutor*).

Monotremata: Australian spiny ant-eater (*Tachyglossus aculeatus*).

The human brains were from subjects of known age but the ages of the animals at death were unknown. Fresh brains were removed from adult specimens of man, macaque monkey, rabbit, mouse, rat, guinea-pig, dog, cat, horse, pig, and sheep, and were fixed in 10 % neutral formalin for 7 days. The remaining material consisted of whole brains fixed in formalin or spirit for unknown periods. Melanin pigment is insoluble in both of these fixatives. The whole midbrain region was removed from these fixed brains, dehydrated and embedded in paraffin wax. Sections 10μ thick were cut transversely through the midbrain in the region of the substantia nigra and then processed according to Pearse's modification of the Masson–Fontana ammoniacal silver nitrate technique (Pearse, 1953). Melanin granules reduce alkaline silver salts and therefore blacken when exposed to ammoniacal silver nitrate. Following fixation in formalin or alcohol, substances that reduce ammoniacal silver nitrate in the cold in less than 24 hr. are most probably melanin, although certain other pigments are capable of reducing alkaline silver salts. Ten to fifteen sections, distributed at intervals throughout the midbrain, were examined in each animal.

RESULTS

Pigment granules are present in at least some of the cells of the substantia nigra in all the animals examined with the exception of the rabbit, mouse, rat, guinea-pig, otter, seal, yellow-backed duiker, hog deer, Indian fruit bat, hedgehog, rufous rat kangaroo, fat-tailed sminthopsis, Tasmanian barred-bandicoot, southern short-nosed bandicoot, Tasmanian ring-tail, Australian spiny ant-eater.

When present, the pigmented cells vary from animal to animal only in regard to their number and intensity of pigmentation. In all other respects the pigmented cells are very similar, no matter which animal is considered. Consequently, the pigmented nigral cells of the macaque will be considered in detail first, since they represent a pattern to be found in all animals containing a pigmented substantia nigra. Then the nigral cells of the other animals will be described.

In the macaque, pigmented cells are seen both in the pars reticulata and pars compacta of the nucleus substantiae nigrae. In both parts a number of cells contain no pigment, but there is no gross morphological difference between pigmented and non-pigmented cells. The nigral cells themselves are fusiform, oval or multiangular in shape, and representatives of all three types contain pigment. The differences in the shapes of nigral cells probably represent no more than differences in the plane of section through multipolar nerve cells, which are clearly visible in thick sections. The pigment itself is composed of discrete granules of roughly uniform size and is confined entirely to the cytoplasm. No granules are visible in the nucleus. The

granules extend for some distance into the axons and dendrites, in fact as far as these processes can be traced. Isolated granules are visible in the surrounding neuropil, but when examined under phase contrast these granules are seen to lie in nerve fibres, and merely represent an extension of the pigmentation into cell processes. The granules are not arranged in any consistent pattern, but are dispersed throughout the cytoplasm of the cells. The number of granules in individual cells varies, and the resultant intensity of pigmentation differs from cell to cell. While some cells are packed with granules, others contain only a few. The majority however, appear to be fully pigmented, in other words contain the maximum number of granules characteristic of this animal.

In all the Primates, the cells of the substantia nigra contain varying amounts of pigment. In none of these animals does the form of the pigmented cells differ from that found in the macaque, but the degree of pigmentation is very variable.

In man, the intensity of pigmentation in the substantia nigra is greater than in any other Primate. The cytoplasm of nearly all cells is pervaded throughout with an amorphous mass of pigment. This aggregation of pigment is composed of granules which are so closely packed together that it is difficult to identify them individually.

In the Pongidae (chimpanzee and gibbon) and the Cercopithecidae (white-collared mangeby, patas monkey, macaque, black and white colobus monkey and baboon), as many cells contain pigment as in man, but the intensity of pigmentation in individual cells is far less. The black pigment granules are distinct, but fewer granules are present in each cell (Pl. 1, figs. 1, 2). In the patas monkey, the pigmented cells in the substantia nigra contain only a few granules, which are finer and not so well defined as in other Cercopithecidae.

In the Cebidae (weeper capuchin monkey, cebus monkey and Humboldt's woolly monkey), the majority of nigral cells contain pigment, but the amount of pigment in each cell is less than in the Cercopithecidae, with the possible exception of Humboldt's woolly monkey, where the nigral cells are heavily pigmented (Pl. 1, fig. 3). In the Callithricidae (lion marmoset and black-tailed marmoset), fewer cells contain pigment than in the Cebidae.

In the Lemuroidea (brown lemur and black-fronted lemur), only a small number of cells of the substantia nigra contain pigment. A greater number of granules are found in the pigmented cells of the brown lemur than in the black-fronted lemur. In the Tupaiidae (tree shrew), a number of pigmented cells are present in the substantia nigra, although these cells do not contain many granules.

In the Lagomorpha (rabbit), no pigment is present in the cells of the substantia nigra. In the Rodentia, no pigment is present in the nigral cells of the mouse, rat, or guinea-pig. In the grey squirrel, the majority of the cells of the substantia nigra are unpigmented, but a few contain one or two fine granules and there is an occasional heavily pigmented cell (Pl. 1, fig. 4).

In the Carnivora, the following animals possess pigmented cells in the nucleus substantiae nigrae: dog, fox, European badger, sand-pig badger, skunk, African genet, Indian civet, domestic cat, and pampas cat. In the otter and seal, the cells of the substantia nigra contain no pigment. In the dog, domestic cat and fox many nigral cells are pigmented, but in no case does the intensity of pigmentation in individual cells approach that found in the Primates (Pl. 1, fig. 5). The disposition of

pigment granules resembles that in the macaque, but there are fewer granules in each cell. In the sand-pig badger, European badger, skunk, African genet, Indian civet and pampas cat only a few cells in the substantia nigra contain pigment. However the individual pigmented cells contain many granules and are as heavily pigmented as those of the dog, domestic cat and fox.

In the Perissodactyla (horse), a number of cells in the substantia nigra contain a pigment that differs from that seen in other animals. This pigment is aggregated into a localized mass, occupying only a portion of the cell, and is not randomly distributed as granules (Pl. 1, fig. 6). In the Artiodactyla, two animals, the musk deer and the sheep, show pigmentation in the substantia nigra. In the musk deer, a number of pigmented nigral cells are present. However, the degree of pigmentation in individual cells does not reach the intensity observed in the Primates, nor indeed that seen in those Carnivora which exhibit pigmentation in the substantia nigra. A few granules are present also in the sheep, though these are finer than in other animals (Pl. 1, fig. 7). No pigment is seen in the nigral cells of the pig, the hog deer and the yellow-backed duiker.

In the Chiroptera (Indian fruit bat), no pigment can be detected in the substantia nigra, nor can pigment granules be seen in the nigral cells of the Insectivora (hedgehog).

In the Xenarthra (six-banded armadillo), a small number of nigral cells contain pigment granules, and the degree of pigmentation in individual cells resembles that seen in the lower Primates and the Carnivora (Pl. 1, fig. 8).

In the Marsupialia examined, two animals, the tree kangaroo and the common ring-tail, show pigmentation in the substantia nigra. No pigment is visible in the nigral cells of the rufous rat kangaroo, the fat-tailed sminthopsis, the Tasmanian barred-bandicoot, the Southern short-nosed bandicoot, and the Tasmanian ring-tail. In the tree kangaroo, a single heavily pigmented cell is to be seen in the pars reticulata of the substantia nigra (Pl. 1, fig. 9), Certain other cells in this nucleus of this animal also contain one or two granules of pigment, but the majority of cells are completely free of pigment. Pigmented nigral cells are present in the common ring-tail, though each cell contains only a few granules.

In the Monotremata (Australian spiny ant-eater), no pigment is present in the cells of the substantia nigra.

DISCUSSION

The intensity of pigmentation in the brain is dependent on age (Foley & Baxter, 1958), and pigment is not present in young animals. Since the ages of the animals investigated in this study were not known, the failure to find pigment in any species does not conclusively indicate that it is never present. Similarly, the number of pigmented cells in any animal depends upon its age. These facts must be considered when evaluating the results of the present study.

Pigment is present in the cells of the substantia nigra in representatives of seven major mammalian orders, the Primates, the Rodentia, the Carnivora, the Perissodactyla, the Artiodactyla, the Xenarthra, and the Marsupialia. With such a wide distribution, this characteristic can be of little or no taxonomic value. Pigmented nigral cell are found most constantly in the Primates and the intensity of pigmentation in

members of this mammalian group is greater than in any other order. Within the Primates there is a progressive increase in the intensity of pigmentation as the relationship to man becomes closer. The maximum intensity is seen in man, where nearly all cells in the substantia nigra contain pigment and where the aggregation of many granules into an amorphous mass is distinctive. The smallest number of pigmented cells and the minimum number of granules per cell is seen in the tree shrew. The Lemuroidae, the Callithricidae, the Cebidae, the Cercopithecidae and the Pongidae show intermediate degrees of pigmentation. In other mammals, there are fewer pigmented nigral cells and each cell contains fewer granules than in the Primates. The pigment granules in lower mammals are finer and less distinct.

The nucleus substantiae nigrae reaches its greatest size and cellularity in the Primates, culminating in man (Kappers, Huber & Crosby, 1936; Brodal, 1948). The increase in size of this nucleus, which probably indicates an increasingly important function, seems to be paralleled by increasing pigmentation of its cells. The nucleus substantiae nigrae is generally considered to be a brain centre concerned with the extrapyramidal control of muscle activity. It is suggested that the presence of pigment in the cells of this nucleus is related to the motor functions of the cells, functions which become increasingly important as the relationship to man becomes closer.

SUMMARY

1. The distribution of pigment in the nucleus substantiae nigrae of mammals was studied in forty-nine species from eleven mammalian orders.

2. Pigment is present in the nigral cells of members of the Primates, the Rodentia, the Carnivora, the Perissodactyla, the Artiodactyla, the Xenarthra, and Marsupialia.

3. Pigment is present in such a wide variety of mammalian orders that this characteristic can be of no taxonomic value.

4. The greatest intensity of pigmentation is found in the Primates, where more nigral cells contain a greater number of larger granules than in other mammals.

5. In the Primates, the intensity of pigmentation increases as the relationship to man becomes closer.

6. The significance of pigmentation in the nucleus substantiae nigrae is discussed.

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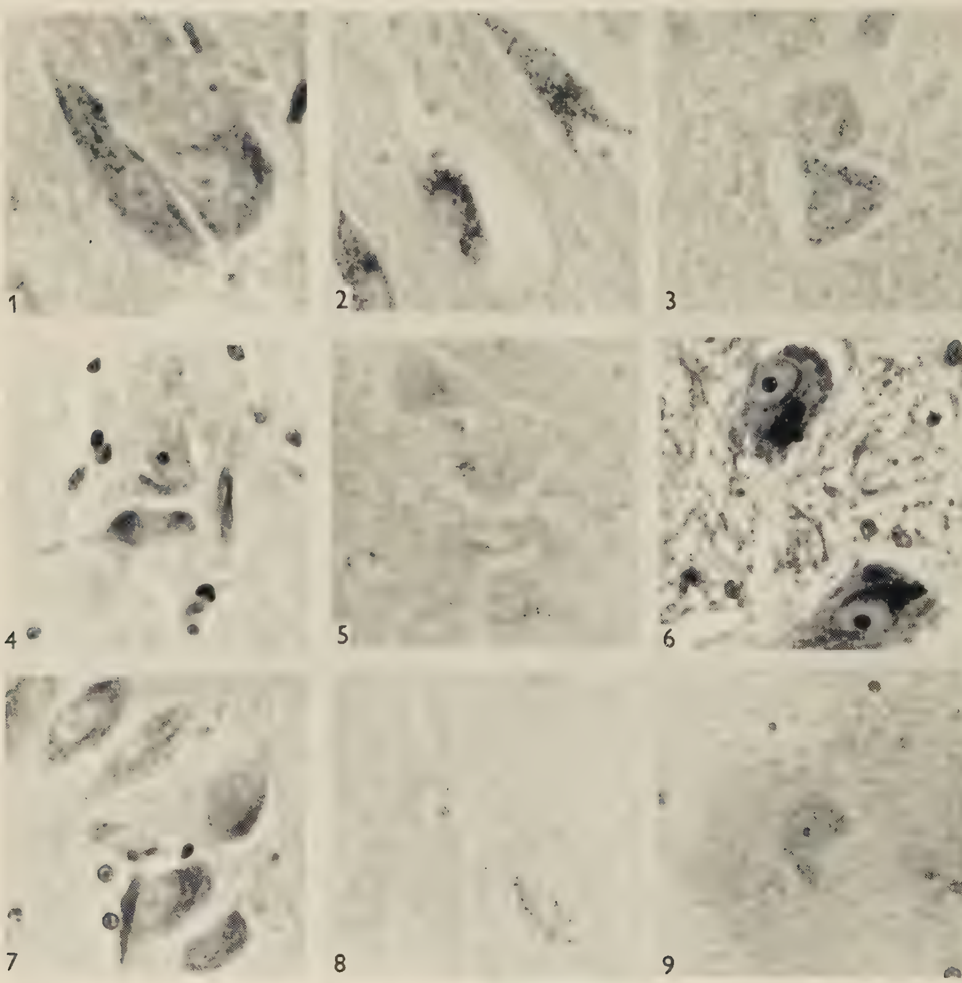

MARSDEN—PIGMENTATION IN THE NUCLEUS SUBSTANTIAE NIGRAE OF MAMMALS

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EXPLANATION OF PLATE

Fig. 1. Pigmented cells of the nucleus substantiae nigrae of the gibbon. Masson–Fontana (×480).

Fig. 2. Pigmented cells of the nucleus substantiae nigrae of the white-collared mangeby. Masson–Fontana (×480).

Fig. 3. Pigmented cells of the nucleus substantiae nigrae of the Humboldt's woolly monkey. Masson–Fontana (×480).

Fig. 4. Pigmented cells of the nucleus substantiae nigrae of the grey squirrel. Masson–Fontana (×480).

Fig. 5. Pigmented cells of the nucleus substantiae nigrae of the fox. Masson–Fontana (×480).

Fig. 6. Pigmented cells of the nucleus substantiae nigrae of the horse. Masson–Fontana (×480).

Fig. 7. Pigmented cells of the nucleus substantiae nigrae of the sheep. Masson–Fontana (×480).

Fig. 8. Pigmented cells of the nucleus substantiae nigrae of the six-banded armadillo. Masson–Fontana (×480).

Fig. 9. Pigmented cells of the nucleus substantiae nigrae of the tree kangaroo. Masson–Fontana (×480).

OBSERVATIONS ON THE ORIGIN OF METRIAL GLAND CELLS IN THE RAT PLACENTA

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The development of metrial gland cells in the pregnant rat was studied by Baker (1948), who traced their origin to mesenchyme-like connective tissue cells in the mesometrial triangle (i.e. the region between the muscle layers at the attachment of the mesometrium to the uterus—Young, 1956), which became basiphilic, rounded and enlarged. He stated that the basiphilia was short-lived, for it declined with the appearance of eosinophilic granules and glycogen. He found cells with the characteristics described not only in the mesometrial triangle but also in the decidua basalis, which led him to conclude that the same stimulus causes the formation of both the metrial gland cells and the decidua. Baker, like Selye & McKeown (1935), who worked on the metrial gland of pseudo-pregnancy, did not mention one of the striking features of metrial gland cells, namely the high frequency of binuclearity.

Our interest in the origin of metrial gland cells was aroused when it was noticed that cells with all the characteristics of metrial gland cells had appeared in the decidua basalis, and also in the ectoplacental cone at a time when none was to be found in the proper site of the definitive metrial gland, the mesometrial triangle. Several features are typical of the fully developed metrial gland cell (Pl. 1, fig. 1). It is a large cell (about 18 to 20 μ in diameter), it is binucleate (Velardo, Dawson, Olsen & Hisaw, 1953) and there is a perinuclear aggregation of acidophilic, PAS-positive, diastase-fast granules surrounded by a rim of apparently clear cytoplasm, which has a high content of glycogen. There are, unfortunately for purposes of certain identification of cell types, numerous cells throughout the pregnant rat's uterus which possess one or other, or even more than one, of these features. It was decided, therefore, in this investigation of the metrial gland in pregnancy, to accept as metrial gland cells only those which were large and binucleate, with acidophilic or PAS-positive diastase-fast granules (according to staining method) as well as a rim of clear cytoplasm. The second type of metrial gland cell (Ellis, 1957) which contains lipid, is usually mononucleate and develops later than the granular type, is not dealt with in this paper.

MATERIAL AND METHODS

In addition to the rat placentae of 12, 14 and 17 days which were examined in a study of carbohydrate materials (Bulmer & Dickson, 1960), there were available also placentae of 9 and 10 days which had been fixed in 10% formalin. This material was serially sectioned at 3, 5 or 7 μ, with the exception of one 10-day specimen sectioned at 10 μ and one 12-day placenta from which sections were cut at 10, 20 and 30 μ. Series of sections extending through each placenta, stained by the trichrome and PAS (with diastase digestion) methods, were examined. The PAS

technique combined with dimedone blockade (Bulmer, 1959) was used for the demonstration of glycogen. Acidophilia of metrial gland cell granules was shown by staining with orange G. The performic acid-alcian blue method of Adams & Sloper (1956) was used to demonstrate protein-bound disulphide groups, a marked positive reaction indicating the presence of at least 4% cystine (Pearse, 1960). Occasional sections were stained for reticulin fibres by the methods of Long (1948) and Gomori (Lillie, 1954). In attempts to demonstrate sex chromatin the techniques of Klinger (1957) and Klinger & Ludwig (1957) were employed.

OBSERVATIONS

At 9 days cells which conform to all the criteria listed above as characteristic of the typical metrial gland cell are situated in the decidua basalis adjacent to the ectoplacental cone (Pl. 1, fig. 2) and, less frequently, in the ectoplacental cone itself. Those in the decidua basalis (Text-fig. 1) are scattered inside a cone, the apex of which is against the apex of the ectoplacental cone. The base of this cone, which will be referred to as the decidual cone to distinguish it from the ectoplacental cone, is in the position shortly to be occupied by the capsule (Bulmer & Dickson, 1961) which will separate the decidua basalis from the inner layer of uterine muscle until this portion of the decidua disappears (Dickson & Bulmer, 1960). The decidual cone is surrounded by the glycogen tissue of the decidua basalis. Approximately in its axis, which coincides with the mesometrial-antimesometrial axis of the uterus, lie the arteries constituting the maternal supply to the developing placenta (Holmes & Davies, 1948). These arteries pierce the inner muscle layer at the centre of the base of the decidual cone.

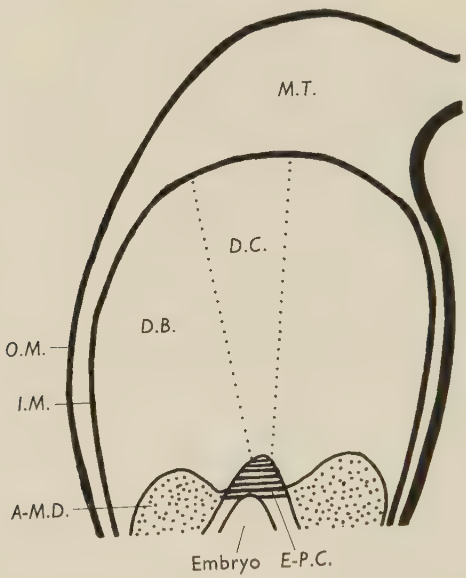
Inside the decidual cone are found decidual cells and typical metrial gland cells. The latter are densely packed at the apex and scattered in decreasing numbers towards the base. It is noticeable that, while all the metrial gland cells in the apical portion of the decidual cone are, like those in the ectoplacental cone, typical, there are towards the base considerable numbers of cells which possess some, but not all, of the features of the typical metrial gland cell. These cells cause difficulty, for it is impossible to be certain whether they should be regarded as developing metrial gland cells or as cells which, since binuclearity and a content of PAS-positive granules are not infrequently to be observed in any region of the pregnant rat uterus, fortuitously resemble metrial gland cells.

At this 9-day stage there is no typical metrial gland cell in the mesometrial triangle, that is, in the connective tissue between the muscle layers at the base of the mesometrium which will later be occupied by the metrial gland. Further, at this time cells possessing even one of the features of the typical metrial gland cell are infrequent in this region.

At 10 days metrial gland cells are common in the ectoplacental cone (Pl. 1, fig. 3) and also in the trophoblastic spur which projects from its apex into the decidual cone in the line of the central artery. This artery, approaching from the maternal side of the developing placenta, stops at the tip of the spur, the cells of which, like those of the ectoplacental cone itself, are bathed by maternal blood without an intervening endothelium. The lack of endothelium makes it difficult, in the case of

occasional metrial gland cells, to decide whether the cell lies in the substance of the trophoblastic spur or ectoplacental cone or whether it lies in the maternal blood bathing these tissues. It seems safe to say that at least the majority of these cells are in the embryonic tissues and not in the maternal blood.

Beyond the termination of the trophoblastic spur the endovascular plasmodium (Duval, 1891; Bridgman, 1948), which, as pointed out by Pritchard (1947), is continuous with the trophoblast, lines a short segment of the supplying artery (or arteries, for there may be more than one). The cells of the endovascular plasmodium



Text-fig. 1. A section illustrating the decidual cone on the ninth day of gestation.

A–M.D.	Antimesometrial decidua	*I.M.*	Inner layer of muscle
D.B.	Decidua basalis	*M.T.*	Mesometrial triangle
D.C.	Decidual cone	*O.M.*	Outer layer of muscle
E–P.C.	Ectoplacental cone		

—the word plasmodium indicates incorrectly that this tissue is a syncytium (Bulmer & Dickson, 1960)—are large and, in common with the trophoblast, which may now be distinguished as junctional or spongy zone trophoblast from the syncytiotrophoblast on its foetal aspect, show intense cytoplasmic basiphilia due to ribonucleic acid (Bulmer & Dickson, 1960) and alkaline phosphatase activity (Pritchard, 1947). Not all the cells in the endovascular plasmodium are typical. Some show a clear rim like a metrial gland cell, some are binucleate and some have perinuclear aggregations of granules which give cytochemical reactions similar to those of metrial gland cells round about. Some are, indeed, indistinguishable from typical metrial gland cells (Pl. 1, fig. 4). All these types occur in the endovascular plasmodium, separated only by the basement membrane from the surrounding concentration of metrial gland cells in the decidual cone. It is noteworthy that there are gaps in the basement membrane.

At 10 days typical metrial gland cells make their first appearance in the mesometrial triangle. They are situated close to the external surface of the inner muscle layer and in close proximity to the central arteries perforating this layer. They are in very small numbers—one or perhaps two in a section. At this stage smooth muscle cells of unusual form may be seen in the vessels further out in the mesometrial triangle. In transverse section (Pl. 2, fig. 5) they are seen to have a central nucleus and a rim from which the cytoplasm appears to have retracted. The cytoplasm concentrated round the nucleus tends to be broken up into small discrete masses or granules. These would seem to be the cells which Selye & McKeown (1935) considered to be one of the types of forerunner of metrial gland cells. They have not, however, been observed to be binucleate and, unlike metrial gland cell granules, the broken-up cytoplasm does not stain with the performic acid-alcian blue technique. Further, arteries with similar muscle cells are to be found in the antimesometrial wall of the uterus, where metrial gland cells do not occur. It is considered that the appearance of discrete masses of cytoplasm simulating granules is artefactual.

The number of metrial gland cells in the decidual cone has, by the 10-day stage, undergone an increase which results in a greater number of them being found in the outer, basal part of the cone.

At 12 days, metrial gland cells are scattered with decreased density through a decidual cone which has enlarged with the growth of the placenta. They occur in all parts of the decidual cone, being seen, at the two extremes, close to the juctional zone trophoblast and near to the capsule (Bulmer & Dickson, 1961). They may also be found, infrequently, among the cells of the maternal glycogenic tissue surrounding the decidual cone. The mesometrial triangle is almost completely filled by a mass of metrial gland cells, which tend to have a perivascular arrangement. The inner muscle layer is disrupted into bands scattered in the inner part of the metrial gland. Not all the metrial gland cells are typical, for some display mononuclearity. Thicker sections of 10, 20 and 30 μ were examined in an attempt to determine whether this mononuclearity was apparent only and due to thin sectioning. Lack of transparency, however, in the thick sections prevented clarification of the problem.

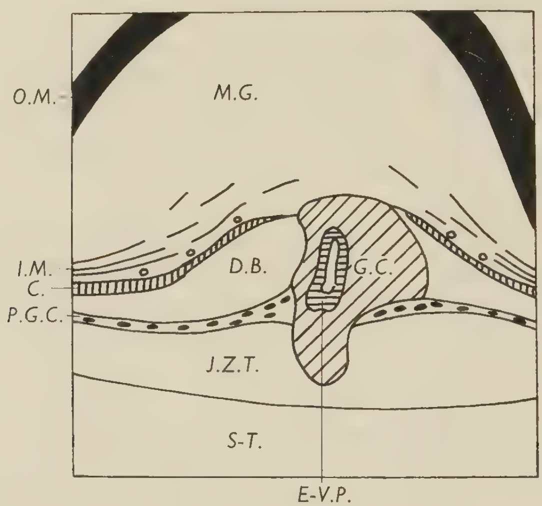
At 12 days all stages intermediate between endovascular plasmodium and typical metrial gland cells are found inside the basement membrane of the supplying arteries (Pl. 2, fig. 6). Occasional typical metrial gland cells are found free in the lumina of these vessels, but they are so few in number that it is difficult to assess their significance. Into this category come also the occasional metrial gland cells in the junctional zone trophoblast. More significance is attached to apparent continuities between endovascular plasmodium and metrial gland cells through breaks —often wide gaps—in the basement membrane of the supplying vessels. These continuities are seen as tongues of endovascular plasmodium projecting into the surrounding metrial gland tissue in the inner part of the mesometrial triangle (Pl. 2, fig. 7). Along the tongue cytoplasmic basiphilia decreases, while the granules, binuclearity and clear periphery of the typical metrial gland cells appear.

At 14 days no metrial gland cell is to be found in the decidua basalis, a finding which, since the decidua basalis is itself disappearing, applies to all later stages. At this time occasional metrial gland cells (indicated by circles in Text-fig. 2) are found at the junction of the decidua basalis with the uterine wall proper, that is, between

the developing capsule (Bulmer & Dickson, 1961) and the disrupted inner layer of smooth muscle which separates them from the vast number of their fellows in the mesometrial triangle.

Continuities between endovascular plasmodium and metrial gland cells in the mesometrial triangle are now even more marked than at 12 days, there being wide openings in the basement membrane (Pl. 2, fig. 8). It can be exceedingly difficult to judge whether a given metrial gland cell is in the endovascular plasmodium of the supplying vessel or in the tissues of the mesometrial triangle outside the vessel.

Many of the cells of the junctional zone trophoblast have become glycogen cells. These cells and the junctional zone trophoblast containing them are separated from



Text-fig. 2. A section illustrating the placenta on the 14th day of gestation. Circles represent metrial gland cells between the capsule and the disrupted inner layer of uterine muscle.

C.	Capsule	*J.Z.T.*	Junctional zone trophoblast
D.B.	Decidua basalis	*M.G.*	Metrial gland
E–V.P.	Endovascular plasmodium	*O.M.*	Outer layer of muscle
G.C.	Cuff of glycogen cells	*P.G.C.*	Placental giant cells
I.M.	Inner layer of muscle	*S–T.*	Syncytiotrophoblast

the decidua basalis, over the whole of the placenta except a small central area, by the layer of trophoblastic giant cells. At the central area the maternal vessels reach the embryonic tissues of the placenta by passing through a hole in the giant cell layer (Dickson & Bulmer, 1960). As mentioned above, these arteries are lined by endovascular plasmodium which is limited externally by a tube of basement membrane. At this stage, the tube of basement membrane no longer extends as far as the embryonic side of the placenta, its end having receded to the level of the capsule. The endovascular plasmodium, which is indistinguishable from junctional zone trophoblast, is not, however, left in contact with maternal tissues by the recession of the basement membrane, for a cuff of glycogen cells is now present, intervening between the endovascular plasmodium and the decidua basalis (Text-fig. 2). A framework of PAS-positive diastase-fast intercellular substance lies among these

glycogen cells. Its continuity with the placental end of the tube of basement membrane suggests that it may be derived from that structure. The appearances indicate that the glycogen cells of the periarterial cuff originate from the endovascular plasmodium and that their outgrowth breaks up the basement membrane. By 17 days, the termination of the tube of basement membrane is in the mesometrial triangle. The cuff of glycogen cells, with their intercellular substance, has advanced *pari passu*, and as a result glycogen cells are now found in the mesometrial triangle. Bridgman (1948) points out that the picture is a confusing one, for it is difficult to determine whether one is dealing with foetal glycogen cells which have grown out from the junctional zone trophoblast or with maternal glycogen cells which, remaining after the disappearance of the rest of the decidua basalis, have migrated into the mesometrial triangle.

An attempt was made, using the techniques for the demonstration of sex chromatin, to discover whether the cells of the outwardly extending cuff are of maternal or foetal origin. There are difficulties in employing the presence or absence of sex chromatin as a criterion for establishing the origin of rat placental cells. First, in the early stages, when the gonad is morphologically indifferent, comparison must be made between embryonic cells which, according to sex, may or may not contain sex chromatin and maternal cells which have no male counterpart. Secondly, reports of the demonstration of sex chromatin in rodent tissues are very few. Klinger (1957) mentions his unpublished findings of sex chromatin in rat nerve cells. In a study of sections of spinal cord of 17-day male and female rat embryos we found that, in each interphase nucleus, three or four bodies could be identified with the appearances and staining characteristics of the sex chromatin described in other mammalian forms. Though counts of male and female nuclei showed that on the average the female nucleus contained one more such body, it was impossible to identify one particular body as the sex chromatin. Examinations of placentae associated with male and female embryos proved similarly unsuccessful. The nuclei of junctional zone trophoblast cells, each with several nucleoli, contained numerous particles resembling sex chromatin. While, on the average, more such particles could be counted in a series of female than in a series of male nuclei, the variation from nucleus to nucleus was so great as apparently to render this an unreliable method for the identification of sex. On the other hand, cells of the mesometrial decidua showed a single intra-nuclear particle situated at either the nuclear or the nucleolar membrane, but it is impossible to be certain that this body is the sex chromatin. There is, nevertheless, a marked difference in nuclear morphology between the trophoblast cells and the cells of the decidua basalis. The glycogen cells of the junctional zone trophoblast and of the outwardly extending periarterial cuff have nuclei which become smaller and more densely staining as the glycogen content increases. They are occasionally binucleate. Many of these cells, however, show nuclei with a morphology similar to that of the adjacent trophoblast nuclei. Intermediate stages can be seen between cells with large granular nuclei, each containing several nucleoli, and cells with small, densely staining nuclei. None of these cells showed any nuclear resemblance to the decidual cells. These findings, such as they are, seem to indicate that these glycogen cells are foetal in origin. Furthermore, the decidual glycogen cells have a supporting network of reticular fibres, which is

absent from the glycogen cells of both the junctional zone trophoblast and the periarterial cuff. At 14 days the few decidual glycogen cells which persist retain their reticulin network but at 17 days, when the decidua basalis has disappeared, there are no longer any glycogen cells with a reticular framework, suggesting that at this stage only foetal glycogen cells are present.

One other observation on the 17-day material is worthy of note. Some of the glycogen cells of the cuff, especially those situated in the region of its advancing edge, that is to say outside the capsule and in the mesometrial triangle, are binucleate and possess granules giving histochemical reactions similar to those of the neighbouring metrial gland cells. A few of these cells are apparently typical metrial gland cells.

DISCUSSION

The observations described above indicate that typical metrial gland cells appear first in the decidua basalis close to the ectoplacental cone and in the cone itself at about 9 days, that at 10 days a very few and at 12 days (when the decidua basalis still contains many) a multitude are found in the mesometrial triangle and that at 14 days none remains in the decidua basalis. Typical metrial gland cells, then, appear from within outwards and disappear from within outwards. The straightforward conclusion to be drawn from these observations is that metrial gland cells are formed in the region of the ectoplacental cone and migrate outwards, formation stopping at about 12 days and migration being complete at about 14 days.

The cell type which is parent to the metrial gland cell is not obvious. At 9 days suspicion must fall on decidua basalis cells and, since metrial gland cells appear in our material as early in embryonic as in maternal tissues, also on the trophoblast cells of the ectoplacental cone. Evidence supplied by later stages supports an embryonic origin, for at 10 days some cells with one or two, and a few cells with all, of the characters of the typical metrial gland cell are found among the cells, generally accepted to be embryonic in origin, of the endovascular plasmodium lining the maternal arteries supplying the placenta. The significant point is that these apparent metrial gland cell precursors are separated from the many mature metrial gland cells in the surrounding decidual cone only by a basement membrane with holes in it. At the 12- and 14-day stages there is even better reason for thinking that endovascular plasmodium gives rise to typical metrial gland cells, for the holes in the basement membrane are much bigger than before and are occupied by tongues of endovascular plasmodium projecting into the metrial gland tissue in the mesometrial triangle. One interpretation of these appearances is that the cells of the tongues gradually lose the characters of endovascular plasmodium and acquire those of metrial gland cells.

It appears that metrial gland cells may be produced by trophoblast which at first lies against the decidua basalis and then grows into the mesometrial triangle, being called in the former situation ectoplacental cone and in the latter endovascular plasmodium. On this view it is not metrial gland cells which migrate outwards but rather their source and another interpretation of their disappearance from the decidua basalis by the 14-day stage is possible—that they are short-lived. It may be reasonable to postulate that they are short-lived because they are polyploid,

and Sachs & Shelesnyak (1955) used the same postulate to explain the short life of deciduoma cells.

Another possible explanation of the apparent outward spread of metrial gland cells is that a substance spreads out from the region of the ectoplacental cone, inducing decidua basalis and mesenchyme cells in the mesometrial triangle to become metrial gland cells. It is difficult to explain on this basis why the main concentration of metrial gland cells is in the mesometrial triangle and not close to the origin of the supposed inducing substance. It is, of course, easy to manufacture hypotheses which circumvent this difficulty but not nearly so easy to obtain evidence which will prove whether the movement is of cells or of an inducing substance.

The apparent origin of metrial gland cells from endovascular plasmodium does not fit easily into a hypothesis of the outward spread of an inducing substance from the region of the ectoplacental cone. The evidence presented in favour of an origin from endovascular plasmodium might sufficiently satisfy a morphological histologist for him to postulate that metrial gland cells can arise from embryonic cells. All metrial gland cells are not embryonic in origin, for it has been shown by Selye & McKeown (1935) that an apparently normal metrial gland develops in association with the traumatic deciduoma of pseudopregnancy. These authors, who did not mention that metrial gland cells are binucleate, stated that they may arise from the endothelial and 'adventitious' cells of vessels, from decidual cells which migrate into the mesometrial triangle and from smooth muscle cells of the uterine wall. They considered that, in fact, no particular mother cell was responsible and that almost any cell in the region could become a metrial gland cell, having first de-differentiated into a cell type resembling a small fibroblast. It must be very difficult to distinguish between a de-differentiated cell resembling a fibroblast and a real fibroblast. Their identification of smooth muscle cells as fore-runners of metrial gland cells is perhaps unreliable. They observed that both typical spindle-shaped smooth muscle cells and round or polygonal metrial gland cells contained glycogen. They also observed glycogen-containing cells of shapes intermediate between the spindle and the round or polygonal and concluded that this represented a change from muscle cells into metrial gland cells. It would seem that these observations merit only a conclusion that the long axes of all the muscle cells are not parallel. Examination of material shows this to be so, after the dispersion of the inner muscle layer during the growth of the metrial gland. We feel that it is impossible to identify a particular cell as a metrial gland cell unless it is large, binucleate and has a perinuclear aggregation of acidophilic or strongly PAS-positive diastase-fast granules. A cell possessing some of these features may be considered a differentiating metrial gland cell, but a cell which possesses none of them cannot possibly be so identified. The adoption of strict criteria may make difficult the locating of the source of metrial gland cells, but it should prevent a problem being accepted as settled when it is in fact unsolved. Velardo *et al.* (1953) adopted criteria similar to ours for the identification of metrial gland cells, although they did not employ the PAS technique. They suggested that the metrial gland cells associated with deciduomata arise from mesenchyme cells. Ellis (1957) also ascribed the source of these cells to mesenchyme cells or alternatively to fibroblasts. He traced the differentiation of the metrial gland cell through stages of cytoplasmic basiphilia, glycogen accumulation and granule for-

mation. The description by Baker (1948) of the development in pregnancy was essentially similar. As indicated above, we are not satisfied that the early stages described by these authors can be accepted without reservation, for there can be no proof that a cell which, in a section, exhibits cytoplasmic basiphilia would have become a metrial gland cell. Neither can there be proof that the cells we describe in our sections as intermediate between endovascular plasmodium and metrial gland cells actually have this relationship to these cell types. We have preferred another characteristic of metrial gland cells, namely, granularity, as an indication of a possible relationship, since cytoplasmic basiphilia is shared by many cell types and cannot therefore be used as a criterion for identification.

While there may be doubts about the recognition of the precise cell types which become metrial gland cells, there is no doubt that in deciduomata granulated metrial gland cells are of maternal origin. One should not, however, take the view, as Selye & McKeown did, that this evidence is sufficient to exclude an embryonic origin for metrial gland cells in pregnancy. There are, as described, appearances which can be interpreted as indicating such an origin. It is fully realized that these appearances can be interpreted in other ways. It is, however, probably not going beyond what is reasonable to say that cells are passing through the holes in the basement membranes of the endovascular plasmodium-lined arteries. We have chosen to suggest that the movement may be outwards, the endovascular plasmodium differentiating into metrial gland cells. Others have suggested that it is inwards. Selye & McKeown, supported by Pritchard (1947) and Bridgman (1948), have suggested that metrial gland cells pass into the endovascular plasmodium-lined arteries and lodge as glycogen cells in the junctional zone trophoblast of the placenta, where their glycogen is released for the nutrition of the embryo. Since we have put forward an opposite hypothesis we must criticize this one, but would emphasize that criticism of one hypothesis and advocation of another are equally sterile when matters involving cell movements have to be considered in sectioned material. One can expect solutions of such problems only by experimental methods, unfortunately of such technical difficulty in this case that a negative finding is more likely to be an indictment of the technique than a revelation of the truth. Selye & McKeown were of the opinion that they had proved the existence of this two-step passage of glycogen from the metrial gland to the embryo, via the junctional zone glycogen cells, when they removed an embryo, but not its placenta, at operation and found that junctional zone glycogen cells accumulated. They gave no information about the methods they adopted to assess the number of glycogen cells before and after operation. If it did occur, an increase would not be evidence that metrial gland cell glycogen reaches the junctional zone glycogen cells but only that junctional zone glycogen cells may be concerned in embryonic nutrition. The observation of Selye & McKeown that junctional zone glycogen cells never contain the granules characteristic of metrial gland cells might seem to indicate that metrial gland cells do not become junctional zone glycogen cells. These glycogen cells are now generally believed to be embryonic in origin (Bridgman, 1948), though this cannot be regarded as proved. Selye & McKeown were apparently firmly convinced that they are not embryonic cells, for they suggested a second maternal source for them—islets of decidua basalis cut off by invading cords of trophoblast. However,

in the rat placenta trophoblast does not invade the decidua basalis but always maintains a plane surface of contact between maternal and foetal tissues (Dickson & Bulmer, 1960). In any event, the 'small cellular' (Selye & McKeown) junctional zone trophoblast should not be held responsible for invading the decidua basalis, since it is separated from the decidua over the whole area of the placenta, with the exception of the central vascular hiatus, by the layer of trophoblastic giant cells.

In short, then, we believe that the view of Selye & McKeown on the metrial gland as a supplier of nutriment to the embryo is, like their view of the origin of its cells, not well substantiated. The only basis for their hypothesis is the finding of metrial gland cells free in the maternal arteries supplying the placenta. We have seen them in this position but, as stated earlier (Bulmer & Dickson, 1960) they are in our material so infrequent that we are not prepared, without more information than is available at present, to attempt to assess their significance. We prefer to present an alternative suggestion, depending on the more striking apparent relationship between endovascular plasmodium and metrial gland cells.

Our suggestion implies that in the metrial gland there are granular cells of both maternal and foetal origins. Examination of all the material used in our studies of the rat placenta, stained by a wide variety of histological and histochemical procedures, does not reveal two types of cell responding differently to any procedure. This may mean that a procedure adequate for the purpose has not been found, that cells of maternal and of foetal origins have so submerged their differences as to seem identical, or that the cells of the metrial gland are all maternal (assuming that the evidence from deciduomata is sufficient to exclude the possibility that all are embryonic). The only evidence in favour of an embryonic origin for some metrial gland cells is the apparent metamorphosis of endovascular plasmodium (which we believe to be trophoblast) into metrial gland cells. It is admitted that this is an interpretation, and that it might be claimed that the evidence is equally suggestive of a metamorphosis of metrial gland cells into endovascular plasmodium. This seems to be a *reductio ad absurdum*, for it would mean that the trophoblast called endovascular plasmodium is of maternal origin. There is no evidence against an embryonic origin, since evidence derived from deciduomata cannot bear directly on conditions in a normal pregnancy. It would seem that the problem is only soluble by direct differentiation between maternal and embryonic tissues in sections by an immunological method, or by demonstrating the growth *in vitro* of metrial gland cells in explants of ectoplacental cone which contain no decidual cells.

The function of the metrial gland remains an enigma. The suggestion by Selye & McKeown (1935) that it is a source of nutrient for the embryo is unsubstantiated. They suspected also an endocrine effect on lactation. Velardo *et al.* (1953) suggested that this activity might be 'to furnish a factor (possibly relaxin) that synergizes with estrogen and progesterone to produce better development of the mammary glands and consequently lactation'. Wislocki, Weiss, Burgos & Ellis (1957) postulated that the acidophilic granules might represent relaxin, though the histochemical grounds for their suggestion were hardly adequate. If some of its cells are embryonic in origin, a luteotrophic activity, direct or indirect, might be suspected in addition to a mammotrophic activity.

SUMMARY

Certain observations suggest that some metrial gland cells in the pregnant rat may be of embryonic origin.

1. Typical metrial gland cells appear as early in the ectoplacental cone as in the decidua basalis and before any are present in the mesometrial triangle, the site of the definitive metrial gland.
2. There is apparently an outward migration of metrial gland cells from the region of the ectoplacental cone to the mesometrial triangle.
3. Developing and fully developed metrial gland cells are found among the endovascular plasmodium, which is believed to be of trophoblastic origin.
4. There are gaps in the basement membranes of the endovascular plasmodium-lined arteries, through which the metrial gland cells developed inside appear to pass outwards into the metrial gland.

Our thanks are due to Prof. R. D. Lockhart for his advice on the preparation of this paper and to Mr A. Cain for assistance with the illustrations.

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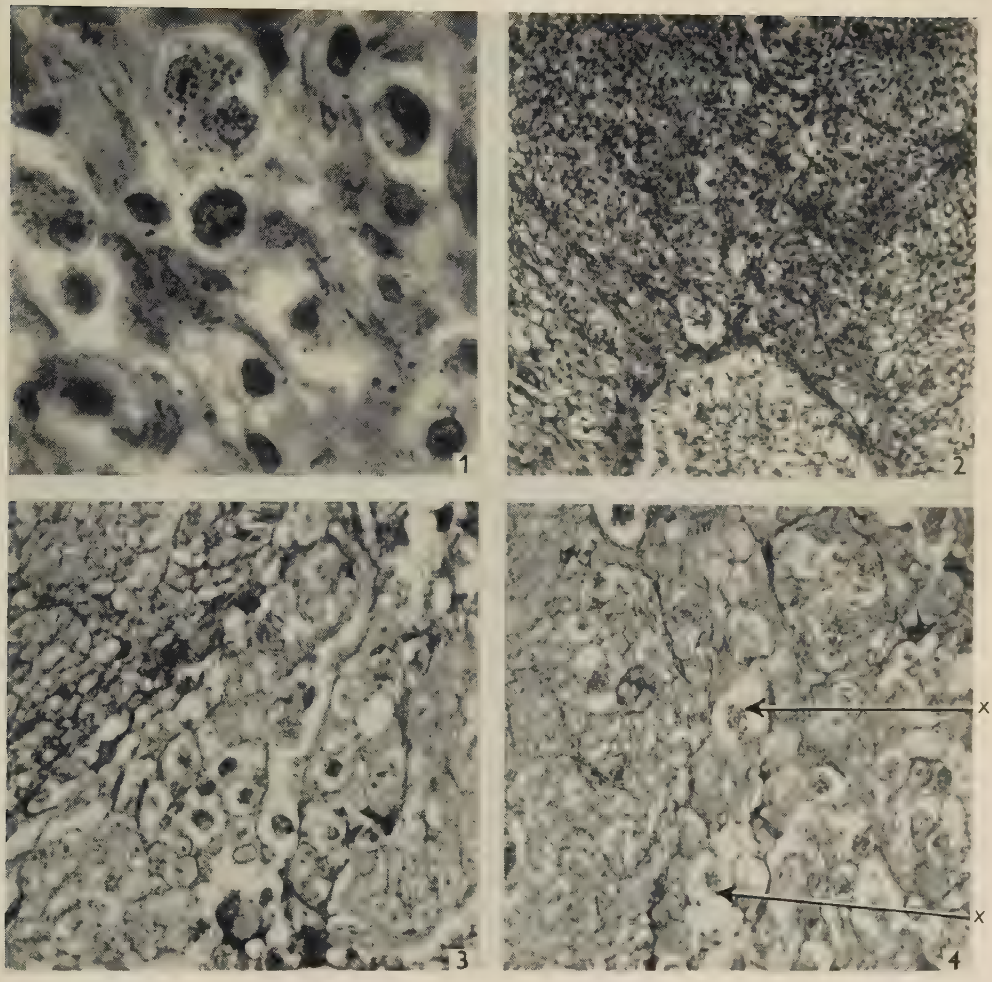

DICKSON AND BULMER—Origin of metrial gland cells in the rat placenta

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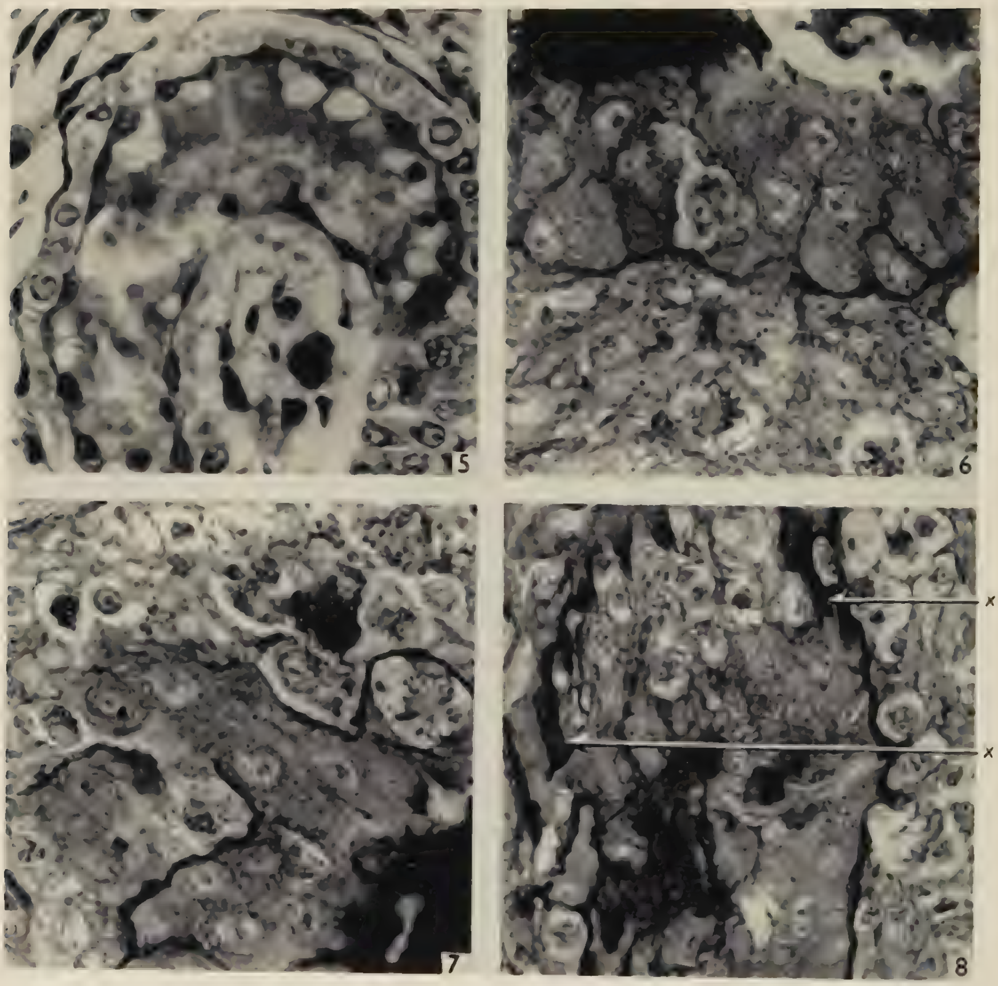

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EXPLANATION OF PLATES

Plate 1

Fig. 1. A metrial gland cell in the mesometrial triangle of a 12-day rat placenta. Trichrome, ×1000.

Fig. 2. The apices of the ectoplacental and decidual cones of a 9-day rat placenta. Trichrome, ×65.

Fig. 3. Metrial gland cells in the ectoplacental cone of a 10-day rat placenta. PAS diastase, ×200.

Fig. 4. Metrial gland cells (x, x) among the cells of the endovascular plasmodium in a 10-day rat placenta. PAS diastase, ×200.

Plate 2

Fig. 5. A cross-section of a blood vessel in the outer part of the mesometrial triangle of a rat on the 10th day of gestation, showing muscle cells with their cytoplasm aggregated into granules. Trichrome, ×350.

Fig. 6. A metrial gland cell lying among the cells of the endovascular plasmodium in a 12-day rat placenta. PAS diastase, ×350.

Fig. 7. A tongue of endovascular plasmodium projecting into the metrial gland through a deficiency in the basement membrane. PAS diastase, ×350.

Fig. 8. A section showing continuity of endovascular plasmodium and metrial gland cells at the 14th day of gestation. The basement membrane is deficient between the points x, x. PAS diastase, ×250.

IN MEMORIAM

JAMES SINCLAIR BAXTER, M.A., M.Sc., M.D., F.R.C.S.I.

Professor J. S. Baxter died in Cardiff on 11 September 1960, aged 55. He was born in Dublin on 4 June 1905, and educated at St Andrew's College, Dublin, and The Queen's University, Belfast. His father was a distinguished lawyer, who, simultaneously, was a Professor in the Law Faculties of Trinity College, Dublin, and of The Queen's University, Belfast. Professor Baxter had a distinguished undergraduate career in Belfast where he obtained the B.Sc. degree with first-class honours, in 1926, and graduated M.B., B.Ch., B.A.O., with honours, in 1929. In his undergraduate days he was very much concerned in student affairs, including the Students' Union, the Literary and Scientific Society and the Officers' Training Corps. His extra-curricular student activities culminated in his election to the Presidency of the Students' Representative Council and membership of the Senate of the University.

After qualifying in medicine, he held appointments as house surgeon and house physician in the Royal Victoria Hospital, Belfast. He then joined the staff of the Anatomy Department of The Queen's University, as a Demonstrator, and at that time had the intention of training for a career in obstetrics. With this objective he studied for, and obtained in 1932, the Fellowship of the Royal College of Surgeons of Ireland. While working for this surgical qualification he wrote a thesis for the M.Sc. degree and also spent a summer in the University of Tübingen, learning tissue culture techniques. His thesis work, on the development of the vagina, and the influence of the late Prof. Thomas Walmsley, so aroused Baxter's interest in embryology and histology that he decided to try to establish a career for himself in anatomy. In 1933 he was awarded a Rockefeller Fellowship which enabled him to spend a year, in Baltimore, in the Department of Embryology of the Carnegie Institution of Washington. There, under the direction of Dr G. L. Streeter and with the stimulus of such members of the staff as Dr Warren Lewis, Dr Margaret Lewis Dr Carl Hartman and Dr Chester Heuser, he received an excellent training in human and comparative embryology. He was also taught a respect for the importance of the details of technique which was to mark his attitude throughout his subsequent career. His criteria of histological excellence were always those he had learned from Dr Heuser; no one who had seen Baxter working at his staining bench is likely to forget his completely preoccupied precision or the clarity, indeed, beauty, of his preparations.

From Baltimore Baxter was invited by Prof. S. E. Whitnall to join the staff of the Department of Anatomy in McGill University, Montreal, where as Assistant Professor of Anatomy he stayed for two years. He then returned to the British Isles, to a University Demonstratorship in Cambridge. In 1939 he rejoined his old chief, Prof. Whitnall, as a Lecturer in the Bristol Department. He was to spend twelve years in Bristol where he was made a Senior Lecturer in 1946 and a Reader in 1949. He was also, for a number of years, Pre-clinical Tutor to the Faculty of Medicine of

PROFESSOR J. S. BAXTER

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Bristol University. In 1946 he was awarded the M.D. degree by that University. With all this background of experience in Anatomy Departments, it had become obvious that Baxter had reached a stage in his career when election to a Professorship might be expected. Consequently his selection, in 1951, to be the successor to Prof. C. M. West in the Chair of Anatomy in Cardiff caused no surprise and much pleasure to his friends. He was to be very happy in Cardiff and he had looked forward to the maturing of the plans, with which he had been so much concerned, for the rebuilding of the Anatomy Department there. At the time of his death he was examiner in anatomy for the Universities of Cambridge and London.

Baxter's research interests lay chiefly in the fields of embryology and histology. His papers on the development of the mammalian urogenital system, which were published in this *Journal* and in the *Carnegie Contributions*, are well known and respected additions to knowledge. His wide knowledge of established histological methods stood him in good stead when he entered the field of histochemistry. In this field his work, in conjunction with Prof. Yoffey, on the adrenal cortex, is also well known. A particularly interesting contribution by Baxter was his demonstration, in 1950, at the International Anatomical Congress in Oxford, of the possession by the germ cells in a 10 mm. human embryo of a very high alkaline phosphatase activity. Unfortunately he never published this work in full but, in the hands of other investigators, the presence of that enzyme has been used most effectively in the study of the distribution of the primordial germ cells.

Baxter was a very good teacher, with a wry sense of humour much appreciated by his students. His teaching experience and his knowledge of the difficulties posed by certain aspects of anatomy and embryology was most useful to him in his editorship of Frazer's *Manual of Embryology*, and of successive editions of his *Aids to Surgical Anatomy* and *Aids to Embryology*. The latter small volume was translated and published in Buenos Aires as *Embriologica Basica*. Many students did, indeed, find this unpretentious volume basic in establishing their knowledge of human development.

Baxter was a devoted member of the Anatomical Society and was very active in its affairs. He had been Recorder and a Vice-President of the Society, and he had served for a long period on its Council and as a member of the Editorial Board of this *Journal*. The visit of the Society to Cardiff, on the occasion of the summer meeting in 1959, gave him great satisfaction and, in spite of a major operation shortly before that meeting, he devoted himself to the well being of our members and guests.

James Baxter was a loyal friend and a most helpful colleague. He was diffident in new surroundings when he gave initially an air of aloofness which deeper acquaintance showed to be no more than an expression of innate shyness. He had great courage in the face of a serious laboratory accident the results of which necessitated a tedious convalescence and much plastic surgery. And in his terminal illness he kept those doubts he must have had about his condition completely to himself.

Prof. Baxter is survived by his wife, whom he married in 1934, and by a son, Dr J. S. R. Baxter. To them both we tender our expression of deep sympathy.

J. D. BOYD

W. J. HAMILTON

REVIEWS

Medizinische Grundlagenforschung: Bd. III. Edited by K. FR. BAUER. (Pp. 754, Figs. 205, Tables 47. DM. 178.) Stuttgart (Georg Thieme Verlag). 1960.

The third volume of this series on basic medical research contains fifteen articles ranging from psychology, through pathology, physiology, biochemistry and anatomy to evolutionary epistemology. They are mostly from Germany, but Britain, Italy, North and South America are also represented. The book is well produced and has both an index of the authors cited in the bibliographies (13 pp.) and one of the varied matter dealt with by them (14 pp.). Excepting that from the U.S., all the articles are in German.

The three most manifestly anatomical contributions are by Barnett & Davies on the structure and function of the synovial tissues; by Schroeder on the capillary realm; and by Asenjo on vascular abnormalities and vascular tumours of the brain. But the articles of skeletal muscle tonus and on its clinical aspects by Göpfert and von Eiff, respectively, fall well within the ambit of modern anatomists. The histologist as well as the biochemist will find useful reading in the complementary articles by Turba & Zillig (*Eiweissmolekül*) and by Schultze & Heide (*Plasmaproteinforschung*). Radioactivity in its useful and its dangerous aspects is dealt with in two papers; the first aspect by Meld, the second by Barth & Frik. Both are very relevant to the anatomy of these atomic days.

The book is dear, falling into the library rather than the personal class. But both the several contributions and their associated bibliographies make it worth having at hand. We all need some such conspectus of other folk's work and opinion. M. A. MACCONAILL

Anatomie des Menschen. Ein Lehrbuch für Studierende und Ärzte. By H. BRAUS continued by C. ELZE. Volume III. *Periphere Leitungsbahnen,* II. *Centrales Nervensystem. Sinnesorgane,* 2nd ed. (Pp. viii+832; 545 illustrations; DM. 69.) Berlin: Springer-Verlag. 1960.

This book combines the third and fourth volumes of the first edition. In the second edition, Elze has maintained the biological approach of Hermann Braus to anatomical problems, presenting them with delightful clarity. Throughout the book much emphasis is placed on functional, developmental and comparative considerations, and use has been made of clinical findings in discussion of anatomical facts. On the microscopical level, however, the book tends to ignore the recent advances in neurohistology, histochemistry and electron microscopy. The Schwann cell sheath of the peripheral nerve fibre is still described as a continuous plasma cylinder (Leitplasmodium), and the satellite cells in the ganglia as plasmatic syncytium. Nerve endings in Merkel's and Meissner's corpuscles are referred to as intra-cytoplasmic, and the terminals in the carotid sinus are shown as a reticulum.

The book is beautifully produced, the illustrations dealing with macroscopical anatomy and the central nervous system are excellent. References are given in the text. There is a comprehensive index for text and illustrations. The book can be warmly recommended to anatomists for its biological approach to the structure of the human body. N. CAUNA

Clinical Anatomy. A Revision and Applied Anatomy for Clinical Students. By H. ELLIS. (Pp. 369; text-figures 205. 37*s.* 6*d.*) Oxford: Blackwell Scientific Publications. 1960.

In the author's words this book attempts to correct the 'unfortunate hiatus between the anatomy which the student learns in his pre-clinical years and that which he later encounters in the wards and operating theatres'. It could be maintained, however, that the pre-

clinical years are better devoted to the study of the body as a whole rather than to acquisition of a catalogue of clinically relevant facts, which are more properly stressed later.

The approach in this book is regional and a brief synopsis of the anatomy of the part of organ, illustrated by line drawings, is followed by a short account of the relevant clinical features. The selection of material is, however, often surprising, and both text and figures contain many factual errors. Some may be mentioned: the relationship between the roots of the brachial plexus and the vertebrae in Fig. 6 is incorrect; on p. 33 there is no mention of the role of the bulbar ridges in the subdivision of the foetal heart; Fig. 32 showing the spermatic cord in transverse section is incorrect; the illustration of the internal anal sphincter in Figs. 49 and 50 is misleading; Fig. 83 shows the origin of Psoas major incorrectly; on p. 143 the glands of Montgomery are incorrectly identified as sebaceous glands; on p. 169 the Extensor digitorum is denied any role in extending the interphalangeal joints; the dermatomes of the upper limb are badly illustrated in Fig. 110; on p. 176 the ulnar nerve is stated to supply the skin of the medial side of the forearm; on p. 222 the superficial inguinal lymph nodes are said to drain only to the deep inguinal nodes; in Fig. 171 the thoracic duct is shown passing behind the vertebral artery; strangely, no mention is made of the pectineal ligament, the pectineal part of the inguinal ligament or of Hesselbach's triangle in the discussion of hernia. On the whole the book can scarcely be said to fulfil the author's aim of providing the clinical student with an accurate synopsis of the essential anatomical knowledge which he requires.

O. J. LEWIS

Primates. Volume 4. *Cebidae—Part A* (*including subfamilies Callicebinae, Aotinae, Pithecinae, Cebinae*). By W. C. OSMAN HILL. (Pp. xxii + 523; 36 plates, 90 text-figures and 9 maps; 189*s*.) Edinburgh University Press.

At first sight the latest volume of Dr Osman Hill's magnificent monograph on the Primates engenders a feeling of amazement that so much is known about a group of Primates that are so notoriously unknown. That on closer study this impression is largely dissipated is no reflexion upon the author who is only too conscious of the gaps that are still to be filled in our knowledge of the Cebidae. Dr Hill, by his own careful dissections of *Cebus* and *Callicebus*, has done much to correct the deficiency, particularly in the sections dealing with splanchnology and myology. In spite of this, the patchiness of our knowledge of these important Primates is very apparent. For instance while myology has been fairly extensively studied in certain muscle groups of certain genera, arthrology is practically unknown; the central nervous system, though far from understood, is better known than the peripheral nervous system. There is no complete account of the reproductive physiology or of the developmental stages of any cebid genus, though placentation has been fairly extensively studied. In the behavioural field, laboratory studies of intelligence are wanting in spite of ample evidence that the intellectual capacity of some cebids is of a high order. This state of affairs stems largely from the fact that New World monkeys have in the past been studied as a means to an end rather than as an end in themselves. Such a criticism equally well applies however to the study of subhuman Primates as a whole. Undoubtedly, too, the paucity of New World fossil Primates has been a factor; palaeontology provides an invaluable stimulus to the pursuance of basic studies of recent forms.

The value of this monograph as a reference book cannot be overemphasized for it contains not only a masterful collation of the world's literature but much original information. Its usefulness, however, would have been augmented by the inclusion where possible of some indication of the *number* of specimens upon which the morphological conclusions of the various authorities are based. Without such an indication it is difficult to evaluate certain observations, particularly in systems where variation is common. Such documentation would, one feels sure, obviate possible misapprehensions that 'all is known' and at the same time engender the spirit of further inquiry that is so badly needed in this field and which Dr Hill has championed for so long and with such authority.

In a review of an earlier volume Prof. Le Gros Clark expressed his regret over Dr Hill's

use of a classification of Primates largely based on Pocock's work. In the present volume the divergencies between the terminology used by Dr Hill and the widely accepted classification of Simpson do not obtrude unduly and certainly do not lead to any confusion, for his classification does not conflict with Simpson's except in respect of *Callicebus*. Pocock placed the titi monkeys in a seperate family, Callicebinae, and this arrangement has been confirmed by Dr Hill on the grounds of the relative primitiveness of *Callicebus* as compared with the night-monkey, *Aotes*, with which form *Callicebus* was grouped by Simpson.

The book is very adequately illustrated with original line drawings, distribution maps and many excellent photographs of living cebids.

J. R. NAPIER

Anatomy: a Regional Study of Human Structure. By E. GARDNER, D. J. GRAY and R. O'RAHILLY. (Pp. xvi + 999, illustrated; £5 5s.) London: W. B. Saunders. 1960.

This new textbook undoubtedly constitutes a landmark in American anatomical literature. The authors have successfully combined within its covers a traditional description of the human body, a review of recent advances, especially in topographical anatomy, and an introduction to the physical examination of the patient.

The first 104 pages, on 'General Anatomy', form a helpful introduction to the human body, provided the beginner reads only the sections in large print. As would be expected in view of the authors' own research interests, the chapters introducing the skeletal system are outstanding. There are concise but fully adequate sections on osteology, surface anatomy and radiology, and the numerous radiographs are uniformly excellent. Although the Nomina Anatomica is used throughout, there is a glossary of the eponyms still in common use in the United States. The illustrations, all in black and white, are clear but have a somewhat dull uniformity.

It is regrettable that there are only scattered references to embryology and no separate sections on histology and neuroanatomy, so that the student would probably require to buy textbooks on all three subjects. These calculated omissions on the authors' part have enabled the book to be sold in this country at a relatively low price, but its lack of comprehensiveness in comparison with some European counterparts is undoubtedly a disadvantage to the student. Nevertheless, this elegant and exciting work can be thoroughly recommended, especially to the professional anatomist and the research worker in related subjects. Its authors are to be congratulated on upholding a scholarly tradition in anatomical teaching. Their book provides convincing evidence that anatomy as a whole, both gross and microscopic, remains 'a living discipline in which research plays an active and significant rôle' as well as one of the foundations upon which the clinical sciences are built.

C. H. BARNETT

Symposia of the Society for the Study of Human Biology. Volume III: *Human Growth*. Edited by J. M. TANNER. (Pp. vii + 120; 48 illustrations + 1 plate; 30*s*.). Oxford: Pergamon Press.

These seven papers are more restricted in their scope than their title or the blurb on the jacket suggests, for they deal almost exclusively with the metrical aspects of skeletal growth. All make interesting reading, and the papers by W. J. Israelsohn and R. M. Acheson, in particular, could serve as a very useful introduction to the subject. The lists of references include recent titles from foreign journals; the only obvious omissions are the names of G. T. Ashley, in connection with the sternum, and H. A. Harris, though the latter is referred to in the text.

Regrettably, the discussion after each paper—stated by the editor to have been 'lively and informed'—has not been included. This omission detracts greatly from the value of this rather expensive book to the ordinary reader, who wishes to know which views were generally accepted and which were challenged by the experts attending the symposium.

C. H. BARNETT

Lymphatics and Lymph Circulation. By ISTVÁN RUSNYÁK, MIHÁLY FÖLDI and GYÖRGY SZABO. (Pp. 7–853; 255 figs.; £7.) Oxford; Pergamon Press. 1960.

New texts dealing with any aspect of the lymphatic system are sufficiently uncommon to merit notice. This text ranges over a wide field, anatomy, physiology, pathology and clinical medicine. After a short, and perhaps somewhat disappointing introduction, there is a brief history of the discovery of the lymphatic system, one which is somewhat recent in comparison with that of other systems but nevertheless interesting, the more so when one probes into the personalities of some of the earlier investigators such as Pecquet.

Chapters on the phylogeny, ontogeny, general and special anatomy constitute much of the first part of the book, special anatomy, which is primarily concerned with the intrinsic organization of the lymphatics in the various organs, constituting the longest chapter. Though still worthy of much further investigation, only limited information concerning the lymph nodes draining the various organs and parts is included. Part two, which is the longest section and occupies close on four hundred pages, is concerned primarily with physiology, including the origin of lymph, role of the connective tissue, absorption, flow and composition. This section contains accounts of the considerable amount of investigation done by the authors at the Medical School in Budapest. Part three is a mixture of physiology, pathology and clinical medicine, dealing with a varied range of topics and, in places, perhaps somewhat controversial. A separate chapter is devoted to chronic lymphoedema and elephantiasis.

There are well over 100 pages of references. Whatever the merits of the text, and these are not inconsiderable, the bibliography is magnificent. One can instance missing references, but these omissions are more than compensated by the wealth of references, particularly to the more recent work done in Eastern Europe and Russia, much of which must be unfamiliar to English workers. The book is provided with an author and subject index. It is written in clear and accurate English and has already been produced in Hungarian, German and Russian.

Whatever criticisms may be levelled against the text, and they cannot be many, this is an excellent and valuable reference book and can be wholeheartedly recommended to those interested in the fundamentals of medical science. In the preface the authors express the hope that the 'work will be kindly received by the reader'. They can rest assured that this hope will be realized.

D. V. DAVIES

The Genesis of the Mouse Skeleton: A Laboratory Atlas. By ZOLTON T. WIRTSCHAFTER. (Pp. xi + 169; 94*s*.) Springfield, Ill.: C. C. Thomas. 1960.

This atlas is similar to an earlier work, *The Genesis of the Rat Skeleton*, by the same author. It portrays the progress of ossification in the skeleton of the mouse as revealed by alizarin stained preparations up to the sixth week after conception. Four complete litters were examined on each day and the times of appearance of ossification centres in each bone are summarized in tabular form. Sex differences are recorded. This atlas will serve as a norm on which other studies of development can be based.

D. V. DAVIES

PROCEEDINGS OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

NOVEMBER 1960

The Annual General Meeting of the Society for the Session 1960–61 was held on Friday, 25 November 1960 at the Anatomy Department, St Thomas's Hospital Medical School, London, S.E. 1.

The President (Prof. J. D. Boyd), Prof. D. V. Davies and Prof. J. J. Pritchard occupied the Chair at the various Sessions.

The following are the authors' abstracts of papers presented.

The mammalian talo-calcaneal articulations. By C. H. Barnett.

St Thomas's Hospital Medical School, London

In man the calcaneus usually has three surfaces articulating with the talus—two distal and one proximal. Often this number is reduced to two, and very occasionally to one. The obliquity of the axis at the subtalar joint was thought by R. L. Jones (*Anat. Rec.* 1945, **93**) to represent 'a relic of our evolution from tree-living pro-anthropoids'. However an oblique axis is present in most of the mammals studied, including the monotremes. In ancestral mammals, talo-calcaneal movements probably allowed cupping of the foot, a movement well seen in the echidna, rather than inversion and eversion of the foot as a whole. It is likely that the calcaneus bore a proximal convex and a single distal concave surface. The former has remained relatively unchanged, but in several mammalian orders the latter has become greatly modified. There has been a tendency for part or the whole of this surface to migrate proximally and become convex, often merging with the proximal surface. The talo-calcaneal articulations then become accessory to the ankle joint, a condition seen in individual members of several orders.

Fibrous epiphyseal plates in mammalian bones. By J. W. Smith.

St Salvator's College, University of St Andrews

In a previous communication to the Society (July 1960), it was suggested that the epiphyseal plates of developing bones conformed, in shape and orientation, to the stress pattern or patterns commonly acting in the bones during activity. In this way shear stress between epiphyses and diaphyses tends to be reduced to a minimum.

It has now been observed that, where an epiphyseal plate lies parallel to the principle tensile stresses and is consequently compressed between an epiphysis and a diaphysis, it is entirely cartilaginous and presents the well known resting, proliferating and calcifying zones. On the other hand in the material examined, any part of an epiphyseal plate which is crossed by strong tensile stresses consists in large measure of coarse bundles of collagen fibres. These fibres do not necessarily lie at right angles to the plane of the epiphyseal plate but are always orientated in the direction of the tensile stress which they resist.

The trabecular pattern of the bone formed at such a fibrous plate differs from the pattern of that formed at a cartilaginous plate, and this is associated with a corresponding difference in the process of ossification by which this bone is formed.

The venous drainage of the pampiniform plexuses of the testes of the rat (*R. norvegicus*). By W. R. M. Morton. *Queen's University, Belfast*

The anatomy of the venous drainage of the pampiniform plexuses in 100 adult male rats has been investigated by visual inspection, by injection of radio-opaque material into the posterior vena cava (PVC), and by radiography. The main vessel draining the plexus is usually a large single vein on each side, but a second vein of equal or near equal size to the first was present in ten cases on the right, and thirteen cases on the left side. On the right side in fifty-nine cases a large vein drained into the right common iliac vein, and in ten of these cases a second vessel entered the PVC; in forty cases a vein drained into the PVC alone, and in one case a single large vein joined the right renal vein. On the left side in ninety cases a large vein drained into the left common iliac vein, in four of these a second vein entered the common iliac just anterior to the first vein, in four cases a second large vein entered the PVC, and in four other cases a second vein joined the left renal vein; in one case two veins entered the PVC only, and in five cases a single large vein entered the PVC; in four cases the drainage was directly into the left renal vein by means of a single large vein. The position of entry of the veins into the PVC could be classified as 'low' or 'high' according to whether they entered below or above the junction of the right iliolumbar vein with the PVC. On the right side twenty-three veins were 'low', and twenty-seven 'high', while on the left side the figures were nine and two respectively. Fine veins draining to the PVC or the renal veins were also commonly present. The labile left iliolumbar vein was observed to join the renal vein in some cases. The significance of the findings will be discussed.

Reversal of the plasma-skimming effect as a result of the differential sampling of the bloodstream by intra-arterial cushions. By Julia Fourman and D. B. Moffat. *University College, Cardiff*

It is well known that in small vessels the cellular constituents of the blood tend to flow in the axial stream, leaving a comparatively cell-free peripheral zone. It has recently been suggested that branches which leave such a vessel at right angles have a plasma-skimming effect since they sample the peripheral stream; the haematocrit value in the branches would thus be lower than that in the parent vessel. The uterine artery of the rat gives off a series of right-angled branches, the origin of each being provided with a pair of intra-arterial cushions which appear to project into the lumen of the main trunk and it seemed possible that these might sample the axial stream, thus reversing the plasma-skimming effect.

To investigate the plasma-skimming effect, samples of blood were taken from one of the vasa recta of the gut and from its parent vessel in twenty adult male Wistar rats and the haemoglobin values of the two samples compared colorimetrically. A similar comparison was made on blood from the uterine artery and from one of its branches in twenty adult female Wistar rats. It was found that in the case of the vasa recta, the orifices of which are not provided with cushions, the haemoglobin value was significantly lower than that in the parent trunk. In the branches of the uterine artery, on the other hand, the value was higher than that in the parent trunk. Since cushions similar to those in the uterine artery have also been found at the origins of the afferent vessels of the juxta-medullary glomeruli in the kidneys of a number of animals, it is possible that this reversed plasma-skimming effect may partly explain the low relative haematocrit in the renal cortex.

Epipharynx (nomen novum). By A. J. E. Cave. *St Bartholomew's Hospital Medical College, London*

Morphologically and essentially the pharynx is the muscular tube connecting oral cavity with oesophagus and is so apparent in non-pulmonate vertebrates. In mammals, however, its roof has become overhung by a purely respiratory chamber situated immediately

posterior to the nasal fossae and itself their physiological continuation, while an opening (isthmus nasopharyngeus) developed in this roof permits respiratory communication between this epipharyngeal chamber and the laryngeal aditus.

The evolutionary history of the parts has been clearly detailed, and various anatomical sequelae have been described, by V. E. Negus (*Mechanism of the Larynx.* London, 1929) and F. Wood Jones (*J. Anat. Lond.*, 1940, 74). In *Homo* the epipharyngeal chamber has lost much of its floor and superficially appears to be a part of the descriptive pharynx: it is therefore traditionally but erroneously termed 'nasopharynx' (worse, lately, 'nasal part of pharynx'), while recently the clinician has dubbed it the 'post nasal space'. None of these terms is satisfactory: the first two do violence to morphological considerations, the third ignores such altogether.

It is therefore suggested that the term 'epipharynx' be adopted instead as a term more indicative of morphological exactitude, topographical precision and etymological convenience in connexion with local pathology.

Tyrosinase activity in the nucleus substantia nigra. By C. D. Marsden. *St Thomas's Hospital Medical School, London*

Black or brown pigment granules are present in certain cells of the adult brain stem of many mammals including man, characteristically in the nucleus substantia nigra and locus coeruleus. These granules have been considered to be melanin; however, their exact nature, method of formation and function remain unknown. Melanin in the skin of mammals is formed from tyrosine by the action of the enzyme tyrosinase. The results of an investigation for the presence of tyrosinase in the pigmented substantia nigra of cats and monkeys was reported and discussed.

The innervation of growing epidermis. By M. J. T. FitzGerald. *University College, Cork*

The development of epidermal innervation has been followed in the pig's snout from 30 to 1600 days after service. Radial growth of the epidermis is associated with a continuous cycle of growth and decay of intra-epithelial nerve endings; tangential growth with ever-increasing nerve fibre populations.

Quantitative studies have shown that a 20-fold postnatal increase in the surface area of the nasal septal skin is associated with a 15-fold increase in its nerve fibre content. The new epidermal nerves are collaterals from the dermal plexus.

Sprouting of axons is known to follow partial denervation of sensory or motor fields. In the present instance sprouting has taken place under physiological conditions; here the requisite stimulus may be a state of *relative* denervation of the epidermis produced by tangential growth.

The influence of acute uraemia upon the survival of skin homografts. By R. G. Burwell, F. M. Parsons and F. G. Smiddy. *University of Leeds*

The effects of acute uraemia upon (*a*) the duration of survival of skin homografts and (*b*) the reactivity of the regional lymph nodes to skin homografts have been investigated in fifty-six adult female rabbits.

In the control series full-thickness skin homografts were applied to the ventral aspect of one ear of each of twenty-three rabbits. Acute uraemia was produced in thirty-three rabbits by removing the left kidney and by clamping the right renal pedicle for 2 hr.; during this time a full-thickness skin homograft was applied to one ear of each rabbit. The animals were killed from 2 to 14 days after operation.

The *mean initial graft rejection time* was prolonged from $5{\cdot}1 \pm 0{\cdot}9$ days in the controls to $10{\cdot}8 \pm 1{\cdot}5$ days in the uraemic animals.

The mean weight increase of the first regional (parotid) lymph nodes on the side receiving the grafts in the control group of animals was maximal on the 6th day. In the uraemic animals the mean maximal rise in blood urea nitrogen occurred between the 2nd and the 4th day after operation, after which it fell at first rapidly and then slowly towards normal. The mean weight increase of the first regional lymph nodes in the uraemic animals was delayed until after the 6th post-operative day and reached a maximum on the 10th day.

In the control group large and medium-sized lymphoid cells appeared in considerable numbers about the 4th to the 8th day after operation in both the diffuse cortical tissue and the medullary tubeculae of each first regional lymph node. These findings agree with those of Scothorne and McGregor (*J. Anat. Lond* , 1955, **89**). The appearance of large and medium lymphoid cells was delayed in the uraemic animals but great accumulations of these cells were found in nodes removed 10 to 12 days after operation.

These observations suggest that acute uraemia may depress immunological mechanisms which occur *principally* in the lymph nodes draining a recently attached skin homograft and which are believed to be responsible for rejection of the graft.

The contribution of migratory epithelium to the healing of trunk wounds in the rabbit. By J. Joseph and Jane Townsend. *Guy's Hospital Medical School, London*

It has been suggested that the epithelium which migrates over a denuded area in mobile skin is only a temporary organ of repair and eventually is lost and that the defect is covered by contraction of the surrounding skin. However some of the migratory epithelium must remain even if repair goes on to completion and the resultant scar is linear. This investigation attempted to estimate the area covered by migratory epithelium 5, 10, 15, 20, 30 and 100 days after a standard wound consisting of the removal of 2×2 cm. of full thickness skin from the chest of a rabbit. Four or five specimens were obtained after each period and the areas covered by migratory epithelium and still remaining denuded were determined by measurement on sections at 0·5 mm. intervals and plotting these measurements on squared paper. It can be shown that the mean area covered by the new epithelium is significantly greater at 10 and 15 days than at 5, 30 and 100 days, and that the mean area covered at 20 days is significantly greater than at 5 and 30 days. It may therefore be concluded that some of the migratory epithelium acts as only a temporary cover for the wound but that 10–15 % of the wound remains covered by this epithelium after 30 and 100 days.

After the standard wound was made it increased in size to a variable extent. It can be shown that the larger the increase in size the greater is the rate of contraction during healing. Thus if one wound is larger than another due to more retraction of its edges, the larger wound will heal relatively more quickly.

Chromaffin mast cells in ungulates. By R. E. Coupland and I. D. Heath, *Queen's College Dundee, University of St Andrews*

Recently Falck, Hillarp & Torp (*Nature, Lond.*, 1959, **183**) described cells in the ox liver capsule and the gut of ox, goat and sheep which gave a positive chromaffin reaction. Later Bertler, Falck, Hillarp, Rosengren & Torp (*Acta physiol. scand.* 1959, **47**) correlated the presence of these cells with the dopamine content of the tissues. The same workers stated that the cells had a specific affinity for the stain nuclear fast red (Gurr) prepared by dissolving 0·1 g. of the dye in 100 ml. boiling 5 % aluminium sulphate and concluded that they were a 'special type' of chromaffin cell.

Three types of nuclear fast red are available commercially. One is a stable diazotate; the second an anthroquinone; the third an amino-azin, nuclear fast red (Herzberg). Only the latter has a specific affinity for granular cells in the ox liver and sheep and ox gut.

In the present work ox and sheep liver capsule and duodenum were fixed in formol-dichromate buffered with M/5 acetate, to pH 6 or 10 % formol with indifferent salts. Frozen, paraffin and carbo-wax impregnated sections were prepared. In unstained preparations cells which contained chromaffin granules were identified in the liver capsule and all layers of the duodenum. The chromaffin reaction was always faint, but was strongest in the sheep's gut. Sections stained with nuclear fast red-aluminium sulphate (NFR-AL) showed that in addition to the chromaffin elements many other granular cells had a specific affinity for the dye. Other staining procedures including toluidine blue and new methylene blue (I.C.I.) indicated that they were mast cells. The affinity of NFR-AL for mast cell granules was verified in a wide variety of different animals. The affinity of the dye for heparin and not dopamine was confirmed by the use of test slides of heparin, dopamine and other naturally occurring amines dissolved in 5 % gelatine which were fixed and stained in the usual way. NFR-AL is a highly specific stain for sulphated mucopolysaccharides including heparin and chondroitin sulphate.

Only a proportion of ungulate mast cells contained chromaffin granules and many in the adjacent areas of the liver or gut failed to give this reaction. The chromaffin-positive cells also gave positive Schmorl ferri-ferricyanide and argentaffin reactions. The cells did not couple with alkaline diazonium salts or give a positive indophenol reaction. These reactions are in keeping with the presence of a catechol. From the assay findings of Bertler *et al.*, it would seem that the catechol is dopamine.

The ontogeny of function of the α-cells of the pancreas of the chick embryo. By T. Adesanya I. Grillo. *St Mary's Hospital Medical School, London*

In the past, the only studies of developing islet cells of the chick embryo were histological. The results were conflicting and not conclusive.

In the present investigation, the problem is studied both histochemically and by means of a specially designed method of biossay. The histochemical method for α cells, by Levine and Glenner (*J. Nat. Cancer Inst.* 1958, **20**) depends on the staining of tryptophan—an amino-acid present in the molecule of glucagon. A strong positive reaction for tryptophan was obtained in both the islet and acinar cells of the pancreas of the 11- and 14-day embryos. It is difficult to draw any definite conclusion from that result.

Extracts were made from pancreases of 11-, 13-, 14-, and 18-day-old chicks as well as from the pancreas of an adult fowl. A new method of bioassay was devised based on the already known *in vivo* effect of glucagon on chick embryonic liver (Grillo, *Proc. Int. Congr. Anat.* 1960: *Anat. Rec.* **136**). The results indicated the presence of a glycogenolytic factor in the pancreases of embryos of chicks from the 13-day-old stage upwards as well as in the adult pancreas.

Islet cell cytology in the normal and cobalt chloride-treated guinea-pig. A fine-structural study. By J. D. Lever and A. C. Esterhuizen. *University of Cambridge.*

Clear distinction can be made between α- and β-cells of the guinea-pig pancreas in light microscopic preparations (stained by the Richardson's phosphotungstic acid-haematoxylin method and the postcoupled benzylidine technique) and also in electron micrographs. Besides a random scattering of single α-cells throughout the islets, there may be collective grouping of these cells especially at the periphery of some islets.

As in other animals the specific secretory granules of the β-cell are less electron dense than the α-cell granules and this is the main cytological point of distinction between the two cell types as seen in electron micrographs. Moreover while the α granules are invariably rounded in outline the β granules may be irregular in shape though for the most part they are also rounded. Both types of granule are individually contained by smooth membranes.

These and other cytological features of the guinea-pig islet cells were described in the normal and cobalt chloride-treated animals.

Chromosome analysis in agenesis of the gonad. By J. E. Gray. *King's College, Newcastle-upon-Tyne*

Agenesis of the gonads leads to the formation of female genitalia which remain immature and the girls possessing them do not develop any signs of puberty. Chromosome analyses of two types of case have been described.

(1) Classical Turner's syndrome who have 45 chromosomes, including only one sex chromsome the X (45/X0) (Ford *et al.*, *Lancet*, 1958, (1).)

(2) Agenesis of ovary without the webbing of the neck and short stature of Turner's syndrome—these have been described as having a complement of 46/XY, i.e., indistinguishable from the male. (Harnden & Stewart, *B.M.J.*, 1959 (ii)).

The chromosomes from a case of type (2) have been analysed and it can be shown that while they may be 46/XY yet the morphology of the chromosomes is such that the chromosome designated as Y may actually be a severely depleted X in this case. A further type (2) case is described, in which the ovary was extremely hypoplastic and chromosome analysis indicated the Karyotype 45/X0.

Further evidence bearing on the increased sensitivity of the haemoglobin of children, pregnant women and patients with malignant disease to oxidation. By W. K. Metcalf. *University of Bristol*

At the last meeting of this Society I showed that the sensitivity of the haemoglobin to methaemoglobinisation is increased in children, pregnant women and in patients with malignant disease. Removal of the carcinoma or termination of the pregnancy causes a rapid return of the resistance to oxidation. In an attempt to determine whether these changes were hormonally governed or were dependent on cell multiplication, the phenomenon was investigated in rats whose growth rates and pubertal changes can be more easily studied.

Rats' blood is no more resistant to such oxidation than is human, and exhibits the same increased sensitivity in the young and pregnant. The changeover in sensitivity was found to be at about 4–5 weeks (50 g.) corresponding to the time of greatest rate of change of the growth rate, whilst the first signs of puberty did not appear until about 7–8 weeks (90–100 g.).

It is thought, therefore, that the sensitivity of the haemoglobin to oxidation is more likely to be related to cell multiplication, rather than to the hormonal changes associated with puberty.

The influence of growth of the teeth and nasal septum on growth of the face. By W. F. Young. *Queen's University, Belfast*

It has been proposed that eruption and growth of the teeth carry the maxilla forwards (Landsberger, *Rep. of Dent. Bd. U.K.*, 1956, and Baker, *Int. J. Orthod.*, 1921, 8). More recently Scott (*Brit. Dent. J.*, 1953, 95) has made the alternative suggestion that the nasal septum, thrusting forwards during growth, carries the upper facial skeleton with it and determines its forward growth.

Experiments were designed to test these hypotheses in growing rats over a period of 20–70 days of age. It was found that neither extraction of molars nor pulpotomy of incisors affected forward growth. However, a large dose of radioactive sulphate, which destroyed the cartilaginous nasal septum (as well as other cartilages), did reduce forward growth of the face. Surgical excision of the septum arrested forward growth completely.

From histological examination it was inferred that the septum could carry the upper facial skeleton forwards by means of firm, fibrous connexions between it and the surrounding bones.

It was concluded that normal forward growth of the facial skeleton is dependent upon normal growth of the nasal septum but not on tooth development.

Growth changes in the cranial base and lower jaw during the closure of the secondary palate in two strains of rat. By J. W. S. Harris. *The London Hospital Medical College, London*

Camera lucida drawings have been prepared from median sagittal sections of rat embryos recovered before and during the closure of the secondary palate. Embryos recovered prior to the onset of closure had a marked angulation of the cranial base in the region of the pituitary canal, which divided the base into an anterior and a posterior portion. During closure of the palate the angulation was gradually obliterated and there was an increase in the length of the cranial base which was mainly due to increased growth of the anterior portion. Longitudinal growth of the lower jaw commenced prior to the onset of closure and before the condylar cartilage developed.

Drawings have also been prepared from sagittal sections of embryos with clefts of the secondary palate produced by treatment with 150,000 i.u. Vitamin A on the 10th or 15th days of pregnancy, and by puncture of the amniotic sac on the 15th day. Different changes occurred in the growth of the cranial base and lower jaw in each experimental group.

The results were compared with the findings in normal embryos.

The intrinsic innervation of the suprarenal gland.
By I. M. S. Wilkinson. *Manchester University*

The intrinsic innervation of the suprarenal gland has been investigated in man, monkey, cat, dog, rabbit, guinea-pig and rat. The nervous elements have been stained by various silver impregnation methods and by supravital methylene blue techniques; nerve-staining has been rigorously controlled using sections stained specifically for reticular fibres.

The profuse innervation of the chromaffin cells of the suprarenal medulla by preganglionic sympathetic nerves has been confirmed in all the species investigated. Each chromaffin cell is in contact with at least one nerve fibre. No terminal anastomoses have been found at the periphery of these preganglionic fibres, although very few apparent nerve-endings have been seen. Intramedullary sympathetic ganglion cells have been revealed in man, but not in any other species.

No evidence of a nerve supply to the cortical parenchyma has been demonstrated. Occasionally a subcapsular plexus and individual fibres traversing the cortex have been observed, but these show no signs of supplying the cortical cells.

The present findings, namely the rich innervation of the medulla and the absence of a cortical nerve supply, are entirely consistent with the embryological mode of origin of this composite gland.

The innervation of the thyroid gland. By W. J. Cunliffe. *University of Manchester*

Satisfactory demonstrations of the nerve fibres in the thyroid glands of man, monkey, dog, cat, rabbit and guinea-pig were obtained using methylene blue, Champy-Coujard and Bielschowsky-Gros techniques. Fundamentally the results in the different species are identical. The thyroid nerves are predominantly post-ganglionic sympathetic fibres. The nerves enter the gland through the superior and inferior hila; most are closely associated with the blood vessels—the vascular nerve plexuses and bundles. The thyroid arteries, especially the medium-sized ones, have a very rich innervation, consisting of two, longitudinally running, interconnected plexuses. The veins have a similar, but less rich innervation. The vascular nerve bundles yield fibres which either augment the vascular nerve plexuses or run a tortuous course amongst the interfollicular cells and around the follicles (the so-called interfollicular nerves). The latter end upon the arterioles and capillaries situated around the follicles.

No evidence of a secretomotor supply was obtained: and so it would appear that the nerves closely control the blood flow and thereby indirectly influence the secretory phenomenon by governing the rate of removal of the secretion.

An experimental study of ascending tracts in the spinal cord of *Lacerta viridis*. By F. Goldby. *St Mary's Hospital Medical School* and L. R. Robinson, *University of Otago and St Mary's Hospital Medical School, London*

Lesions have been made in the spinal cord (partial transections) and posterior nerve roots have been cut in a number of specimens of *Lacerta viridis*. After intervals varying between 8 and 38 days the resulting ascending degeneration has been studied in sections prepared by the Holmes and Nauta (Guillery's modification) silver techniques. The principal result has been to demonstrate a compact ascending tract consisting of fine fibres of fairly uniform calibre approximately in the position of the fasciculus gracilis of mammals. More scattered ascending fibres are also present in the superficial parts of the lateral columns where they are most numerous close to the entering posterior roots. It appears, therefore, that the position of ascending fibres in the reptilian spinal cord is generally similar to that in mammals. The results were illustrated and further details discussed.

Electron microscopic observations on regenerating nerve. By A. A. Barton. *Royal College of Surgeons of England*

An examination of the distal portion of the rabbit's sural and peroneal nerve during Wallerian degeneration is being undertaken with the electron microscope. Certain preliminary findings, 2 cm. below the site of section, seem to be of interest. Fourteen days after section collagen fibres are closely associated with the Schwann cell and evidence suggests that this is produced from these cells.

The contents of the Schwann cell at different intervals of time after section was discussed. At 14 days they contain droplets of electron dense material which sometimes exhibit concentric bands similar to those exhibited by phospholipid fixed in osmium tetroxide. These cells also contain what is thought to be the remains of the myelinated fibres. Their association with new nerve fibres at different intervals of time was shown and the relationship between nerve fibres and 'Schwann tubes' discussed.

Some effects of axon severance in cholinergic neurones of *Xenopus* larvae. By P. R. Lewis and A. F. W. Hughes. *University of Cambridge*

In the late-stage tadpoles of *Xenopus laevis* the musculature of each hind limb is innervated by a discrete group of ventral horn cells which are strongly stained by the thiocholine technique for true cholinesterase. These cells show a typical chromatolysis from 3 to 5 days after ablation of the limb, but this chromatolysis is not associated with any diminution in enzyme activity. During the succeeding week, however, the enzyme activity of many of the cells becomes much reduced and the reappearing Nissl substance often takes up the form of a dense perinuclear ring: such abnormal cells eventually degenerate and disappear. In the nerve trunk supplying the hind limb a proportion of the fibres stain for true cholinesterase. Following limb ablation, this staining becomes much more intense and individual fibres become noticeably distended. Accumulation of enzyme in the severed axons is detectable near the cut ends as early as 6 hr. after operation and after 3 days extends for as much as 1 mm. back towards the spinal cord.

Cholinergic afferent pathways to the hippocampal formation and amygdaloid in experimental material. By C. C. D. Shute and P. R. Lewis. *University of Cambridge*

The cingulum (medial portion), dorsal fornix, alveus, fimbria and stria terminalis (lateral portion) of the rat contain fibres which stain by the thiocholine method for acetylcholinesterase. The strial fibres can be traced from the bed nucleus of the stria terminalis to the amygdaloid. The remaining fibres appear to arise from the medial septal nucleus, nucleus of the diagonal band and probably the interstitial nucleus of the ventral hippocampa

commissure, and to be afferent to the hippocampal formation. Section of any of these tracts causes AChE to pile up in the axis cylinders of damaged neurons on the cell body side of the lesion, and to disappear on the other side. This phenomenon provides a useful method of determining the polarity of cholinergic tracts. Lesions involving the alveus and fimbria cause AChE to disappear from the hippocampal formation where it normally forms characteristic bands, and from layers i and iii of the cingulate gyrus. The findings were discussed in relation to the afferent innervation of the hippocampus and to the physiology of cholinergic neurons in the central nervous system.

The myenteric plexus in the stomach—an experimental study. By J. R. Rintoul. *St Salvator's College, University of St Andrews*

Unilateral cervical vagotomies were performed on a series of experimental animals including eleven rats, nine guinea-pigs and three rabbits. These animals were sacrificed after varying postoperative periods (4 hr. to 14 days), after which the stomachs and other portions of the alimentary tract were removed and fixed together with identical segments of bowel from control animals, preliminary to staining with a modified Bielschowsky-Gros silver impregnation technique.

Examination of Auerbach's plexus in frozen sections of the stomachs of the experimental animals revealed the presence of pear-shaped argyrophilic swellings in the vicinity of Type II (Dogiel) neurons. These swellings, which were interpreted as degeneration products of preganglionic vagal terminals, were not observed in the gastric myenteric plexus of the control animals. The number and size of the argyrophilic swellings was found to vary with the length of the postoperative survival period and was most prominently demonstrated in the stomachs of rats which had survived 5–8 hr. after operation.

A new theory was proposed to explain the mechanism of production of these argyrophilic swellings.

Electron microscopic evidence for a relationship between dense bodies and mitochondria in motor nerve cells. By G. Hudson* and J. F. Hartmann. *University of Minnesota, U.S.A.*

Although dense bodies ('microbodies') have been noted in the cytoplasm of various types of cell, views as to their functional significance appear conflicting.

In an electron microscopic study of the hypoglossal nuclei of twenty rabbits examined 4–16 days after unilateral hypoglossal neurotomy, dense bodies were observed in the cytoplasm of motor neurons of both sides, there being on average about one dense body to every four mitochondrial profiles. The simplest form of dense body showed a granular structure without a limiting membrane; others showed a limiting membrane (with either one or two dense layers) and varying degrees of internal organization in the form of double membranes. In some of the more highly organized structures, electron-transparent areas were evident. The appearances were consistent with the possibility that different forms of dense body represent developmental stages leading to or from mitochondria.

The preliminary results of a quantitative study, in which counts were made on the micrographs, confirmed that mitochondrial numbers were increased following axonal section (Hartmann, *Anat. Rec.* 1954, **118**), and showed that there was an associated increase in the number of dense bodies. This may be interpreted as evidence in favour ot the developmental relationships of dense bodies to mitochondria.

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The morphology and innervation of segmental muscle in the lamprey *(L. fluviatilis)*. By A. Peters and B. Mackay. *University of Edinburgh*

The segmental muscle of the lamprey (*L. fluviatilis*) has been studied by silver staining, cholinesterase staining, and electron microscopy.

Silver stained sections show that a myotome is composed of horizontally arranged units, each of which extends throughout the myotome from its lateral to medial, and cephalic to caudal extents. A myotome is built up of a series of such units stacked one above the other. A unit consists of six horizontal layers. The dorsal and ventral layers are composed of longitudinally arranged muscle fibres (parietal), and where the myotome abuts on the skin, these two layers are continuous with one another around the lateral edge of the central four layers. Each of the central four layers is in the form of an individual sheet of muscle surrounded by its own sarcolemma. Although the myoseptal margins of these sheets may be crenated, there is no evidence of their subdivision, and each must therefore be considered as a single muscle plate.

Cholinesterase studies and electron microscopy show that while the central muscle plates are innervated in their myoseptal regions only, the parietal fibres may be innervated at any point along their length.

Pinocytosis in osteoclasts. By N. M. Hancox. *University of Liverpool*

In fixed tissue sections of decalcified bone, osteoclasts often contain cytoplasmic vacuoles. Well known in the literature, they have been investigated in recent times particularly by Kroon; sometimes they appear empty, whilst at other times they contain material staining like the adjacent bone. Their general appearance and distribution in the cell suggested to the present author that they might be pinocytic in origin.

Motion picture studies have been carried out upon osteoclasts *in vitro* and, as part of the communication, representative film sequences were projected. These showed that osteoclasts do indeed pinocytose vigorously. Whilst, of course, this does not prove directly that the vacuoles seen in sectioned cells originate in the same way, it makes this explanation very likely.

The possible functional significance of pinocytosis in bone absorption was discussed briefly. In electron micrographs obtained in this laboratory it has been possible to identify aggregates of bone salt crystals, particles of apparently unaltered bone, and bits of collagen fibrils within the vacuoles; the latter are thus evidently involved in 'cytosis' of loosened bone materials. Another possibility is that on discharge some of the vacuoles may release enzymes which act upon the bone.

The staining of sulphated mucopolysaccharides in tissue sections. By I. D. Heath. *Queen's College Dundee, University of St Andrews*

Sulphated mucopolysaccharides occur in the form of heparin, in mast cell granules and as various isomeric chondroitin sulphuric acids in cartilage ground substance, mucins, skin and other sites (Stacy, 1946, *Advances in Carbohydrate Chemistry*, **2**), the concentration varying from tissue to tissue. These have been investigated using a variety of dyes which were prepared by a special technique.

Ox trachea and rat skin were fixed in formol-dichromate and formol-calcium respectively. Paraffin sections dewaxed in the usual manner were stained for 5–10 min. in a 1 in 3 dilution, with 5 % aluminium sulphate, of stock dye solution. The dye solution was prepared by dissolving 0·1 g. of dye in 100 ml. of boiling 5 % aluminium sulphate. In all fifty-three dyes were tried, most were basic in nature, but some were acidic. Test-slides of heparin (ox, pig and sheep), histamine, 5-hydroxytryptamine and 5-hydroxytryptophan were also prepared and stained as above.

Mast cells granules and cartilage ground substance were stained deeply with basic dyes; in some cases an orthrochromatic reaction occurred while in others metachromasia was observed. Mucins and skin have less affinity for the dyes. On test-slides only the heparin reacted.

The quinone-imine group of dyes gave the best results, toluidine blue, methylene blue, neutral red and nuclear fast red (Herzberg) being the best of these. These dye-solutions

prepared as above are simple to use and are highly specific for staining sulphated mucopolysaccharides. They are particularly useful for the detection, identification and, when necessary, enumeration of mast cells.

Observations on the reactions of phosphotungstic and phosphomolybdic acids with tissue sections. By D. BULMER. *University of Aberdeen*

The effects of blocking techniques on certain histological methods which involve the use of phosphotungstic acid or phosphomolybdic acid have been investigated. Particular attention has been paid to the differential staining of muscle and collagen by Mallory's phosphotungstic acid-haematoxylin.

Methylation or brief alkaline hydrolysis converts the normal blue-staining of smooth muscle with PTAH to red, though this effect is reversed by interposing an exposure to mercuric chloride or chromic acid before staining. Acetylation and phosphorylation block the staining of muscle on short exposure to PTAH but longer exposure reverses the blockade. Deamination impairs the staining of muscle to a greater extent than that of collagen, while benzoylation abolishes staining of both. Corresponding effects are produced on the staining reactions with Mallory's phosphotungstic acid-aniline blue-orange G mixture and on the binding of phosphomolybdic acid to tissue sections demonstrated by the molybdenum blue reaction.

The differential staining with PTAH cannot be firmly correlated with particular reactive groups. The effects recorded above are probably due, in part, to interference with the internal bonding of the protein molecules, permitting or preventing the entry of different polyacid-dye complexes.

Histochemical observations on cholinesterase activity in the autonomic ganglia of man and mammals. By N. THAKAR NAIK and N. CAUNA. *King's College, Newcastle upon Tyne*

Sympathetic and parasympathetic ganglia have been investigated histochemically in man, cat, rat, guinea-pig, mole and hedgehog using a modified Koelle's technique. The tissues were fixed in neutral 10% formalin from $\frac{1}{2}$ to 18 hr., and frozen sections were incubated from 10 min. to 46 hr. at pH 4·4–7·0. Samples of the same tissues were stained for nerves by a simplified Bielschowsky-Gros silver method.

It was found that the distribution of cholinesterase in the autonomic ganglia showed a wide range of species differences. In the sympathetic ganglia of the cat the majority of nerve cells gave a negative reaction with acetyl substrate, but in the other species examined all the nerve cells were positive in varying degrees. In human lumbar ganglia dendrites as well as cell bodies gave a positive reaction. With the butyryl substrate sympathetic nerve cells gave a negative reaction in most species. In the ciliary ganglion of the cat nerve cells and their dendrites gave a positive reaction with acetyl substrate but a negative reaction with butyryl substrate.

The gut wall of man, cat, rat and hedgehog showed both positive and negative nerve cells and the ratio of the two types varied from one part of the gut to another and from species to species.

Birefringence phenomena and staining differences of cellular nuclei and other structures in histological sections. By J. KRUSZYNSKI. *University of Liverpool*

It is well known that with many techniques cell nuclei and erythrocytes do not stain with equal depth. Some authors have claimed that this reflects differing functional states of cells, others have suspected artefacts. The problem has been pursued by the present author.

Different fixed tissues, stained and unstained, have been examined in transmitted, phase contrast and polarized light. It has been found that the staining and optical differences of

some cells are connected with the condition of their fixed cellular and nuclear membranes. Membranes of erythrocytes and some cell nuclei show a relatively low and selective permeability to different reagents like multichrome stains or hydrocarbons. So-called deparaffinized sections may still contain paraffin particles. It has been shown that the same phenomenon also occurs with other structures such as the fixed cytoplasm, brush borders, granules, chromosomes, nucleoli or elastic and collagen fibres.

This peculiarity may affect the birefringence of anisotropic structures, alter the refractive index of fixed tissues and form an important source of error in phase-contrast, interference microscopic and histochemical studies.

The effect of section of the testicular blood vessels in immature rats *(R. Norvegicus)*. By W. R. M. Morton. *Queen's University, Belfast*

Fifty-four rats aged 14–35 days at the time of operation were used. In group (*a*) the testicular artery to fifty testes, in group (*b*) the testicular artery and main vein from the pampiniform plexus of thirty-one testes, and in group (*c*) the main vein from the pampiniform plexus of twenty-nine testes were sectioned between ligatures. Fifty-three of the animals were successfully mated to normal females with the production of offspring, between the 72nd and 140th days of age. One animal in group (*a*) inseminated three females without fertilizing them. Post-mortem inspection and X-ray examination of the vascular arrangements following the injection of 'Micropaque' showed that in group (*a*) sixteen testes were supplied by the vaso-epididymal arterial loop (VEL) alone, nineteen testes by the VEL supplemented by new anastomotic vessels across the site of section, and thirteen testes predominantly by the anastomotic vessels (two not included). In group (*b*) seventeen testes were supplied by the VEL, and seven testes by the VEL supplemented by anastomotic vessels (seven not included); and in group (*c*) the vasal vein drained the pampiniform plexus in three cases, and the vasal vein and small veins from above the point of section in seven cases (seven cases not included). Histological examination showed that in group (*a*) twenty-seven testes had some degree of degeneration, twenty no degeneration (three not included); in group (*b*) seven showed degeneration and twenty-two no degeneration (two not included), and in (*c*) seven showed degeneration and twenty-two no degeneration.

THE UPTAKE OF TRITIUM-LABELLED THYMIDINE BY LYMPHOID TISSUE

By J. M. YOFFEY, W. O. REINHARDT, and N. B. EVERETT
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In 1885 Flemming, in one of the first studies of mitotic division in mammals, described in lymphoid tissue a nodular formation consisting of a clear central portion surrounded by a dark rim. The central portion he described as a germinal centre, because of the numerous mitoses which it contained. The daughter cells after division would be pushed—or migrate—outwards to form the peripheral darker zone of small lymphocytes which then entered the lymph sinuses, and were carried in the lymph stream to the blood. Flemming described germ centres in lymph nodes (ox and rabbit) Peyer's patches (rabbit) and lingual tonsils (man). The light central portions of the nodules had been noted previously by Brücke (1854), His (1862—he termed them 'Vacuolen'), and Frey (1874), but their significance had not been appreciated.

Flemming's view of the germ centre held the field for many years. (For a review of the earlier literature see Latta, 1922.) Latta (1921, 1922) seems to have been one of the first to challenge it seriously. He thought it was a region of degeneration—more especially in the spleen—consequent upon impairment of the blood supply to the central portion of large nodules. Hellman (1939, 1943) termed the germinal centres 'reaction' centres, regarding them as areas of reaction to noxious substances reaching them via the blood stream.

Maximow (1927) came to the conclusion that the germinal centres were structures which showed cyclic changes, and this view was substantiated and amplified by the later work of Conway (1937). When cell division was active, numerous mitoses could be seen. During the time when activity was at a low ebb there was no sign of cell formation, and it was the appearances at this stage which suggested degenerative changes. The position has been complicated by the fact that in some animals, e.g. cat (Röhlich, 1928), monkey (Taliaferro & Cannon, 1936) and rat (Kindred, 1938) the germinal centre may consist of two distinct zones, an inner active portion next to the medulla, and an outer inactive portion near the capsule.

In recent years still other views have been put forward concerning the structure and function of the germinal centre. Thus Trowell (1957) and Hamilton (1957) have suggested that there is considerable re-utilization of lymphocytes, ingested by the macrophages which are often a conspicuous feature of the germinal centres. These macrophages may be quite large, and their pale cytoplasm contains dark-staining bodies, noted by Flemming (1885) and termed by him 'tingible Körper'.

Yet another interpretation is that advanced by Ortega & Mellors (1957), who thought, following some earlier observations of Leduc, Coons & Connolly (1955) that the germinal centres were glandular in function and produced gamma globulin.

The various views which have been put forward concerning the germinal centres fall essentially into two main categories. According to one group of observers, the germinal centre is a region of active cell proliferation, whereas according to the second view it is not. The evidence for cell multiplication rested initially upon the observation of mitoses. More recently it has been provided by the uptake of radio-active precursors into newly synthesized DNA (e.g. Andreasen & Ottesen, 1945; Yoffey, Hanks & Kelly, 1958; Walker & Leblond, 1958; Cronkite, Bond, Fliedner & Rubini, 1959; and many others). The site of such uptake can be identified in radioautographs of sections. In the present work we have endeavoured to study the extent of cell multiplication by the use of tritium-labelled thymidine.

A discussion of the significance of labelling after the administration of tritiated thymidine, and a report on the appearance of labelled cells in thoracic duct lymph and blood will be found elsewhere (Everett, Reinhardt & Yoffey, 1960). Briefly, it may here be stated that tritiated thymidine is incorporated rapidly and specifically into DNA which is being synthesized prior to mitosis. Cells which take up thymidine are therefore growing cells which will subsequently divide.

MATERIAL AND METHODS

The work has been performed on 15 male guinea-pigs, of a mixed strain, weighing approximately 400 g. Observations have already been published on the appearance of labelled cells in the thoracic duct lymph of these animals (Everett, Reinhardt & Yoffey, 1960).

Tritiated thymidine (Schwarz), with specific activity of either 0·36 or 1·9 c./m-mole was given intraperitoneally or intravenously in doses of 1 μc./g. body weight and at varying intervals afterwards under sodium pentobarbital anaesthesia (4 mg./100 g. body weight) the thoracic duct lymph was collected for 1–3 hr. and the animal then killed. Samples of thymus, spleen, mesenteric lymph nodes and Peyer's patches were fixed in 10 % formol saline or Bouin's fluid, and after paraffin embedding were cut at 6 μ. In addition, suspensions were made of the cells of the thymus and mesenteric lymph nodes by cutting these tissues into small pieces and teasing in homologous serum. Smears were made of these suspensions and fixed in absolute methyl alcohol for 4 min. The tissue sections and the tissue smears were stained in haemalum for 2 hr. and in 1 % eosin in 70 % alcohol for 5 min. Subsequent to the transfer through 95 % and 100 % absolute alcohol the slides were dipped in a 1% solution of nitrocellulose in ether alcohol, dried in air for 30 min., and dipped a second time. They were then dried overnight at 37° C. Coated radioautographs were then prepared as described previously (Everett, Reinhardt & Yoffey, 1960) using NTB-3 emulsion (Eastman Kodak). The slides were exposed for 2–5 weeks and developed as previously described (*loc. cit.*). The radioautographs of the tissue smears, after washing, were stained once more using a 0·5 % Giemsa solution in phosphate buffer of pH 6·4 at 5° C. for 12–15 hr.*

* Radioautographs are now prepared of tissue smears without the coating of nitrocellulose and without staining with haemalum and eosin. These preparations, after development, can be stained in a few minutes with conventional blood stains (Everett, Rieke, Reinhardt & Yoffey, *Ciba Symposium on Haemopoiesis*, 1960).

RESULTS

Sections. Labelling is most intense before any of the labelled cells have begun to divide. The best autographs are therefore those obtained within the first few hours after giving thymidine, before mitosis causes dilution of the label.

Pl. 1, fig. 1, shows the subcapsular sinus and adjacent cortical tissue of a mesenteric lymph node 4 hr. after the administration of thymidine. The most heavily labelled cells, one of which is in the sinus, are large lymphocytes. Some labelled medium lymphocytes may also be seen. The appearances suggest a steady migration of large lymphocytes into the sinus. Pl. 1, fig. 2, from the same gland, shows a germinal centre in which most of the cells are labelled. Pl. 2, fig. 3 on the other hand, shows a nodule in which considerably fewer cells are labelled—though those which are labelling do so intensely—and the germinal centre is much less active than that depicted in Pl. 1, fig. 2. Germ centres in different parts of the gland may show great variations in their proliferative activity.

Pl. 2, fig. 4, is from a Peyer's patch of the same animal, and shows a fair number of labelled cells. The lymphoid tissue of Peyer's patches is usually the site of proliferation which may be a good deal more active than shown in Pl. 2, fig. 4. Pl. 2; fig. 5, is from the spleen, and shows active proliferation, with many large lymphocytes labelling heavily.

Table 1. *Number and size distribution of labelled cells in mesenteric lymph node of guinea-pig at varying times after administration of tritiated thymidine*

No. of animal	Hr. after thymidine	No. of cells counted	Labelled cells, % of total	Size distribution of labelled cells				
				Large	Medium	Small	Damaged*	Total
100	0·5	2018	4·0	11	55	0	14	80
101	1	4122	3·8	28	83	7	38	156
94	3	4029	3·8	27	98	17	10	152
88	4·75	5132	3·0	20	105	17	12	154
89	5	4080	5·7	20	131	50	30	231
103	7	6189	4·4	32	148	53	42	275
106†	7	4037	6·8	14	103	53	103	273
104	12	6164	6·6	0	97	289	21	407
90	14·5	5130	5·5	11	111	134	27	283
105†	17·5	5091	3·7	24	88	38	36	186
92	27	3054	6·7	7	46	116	35	204
95	52·5	4100	11·7	1	62	401	17	480
91	78	4043	9·0	5	84	243	30	362
93	14·3	4036	15·3	0	59	540	20	619
96	190·5	4057	8·9	0	31	326	3	360

* Some of the damaged cells could be identified as reticulum cells, others as lymphocytes in different stages of disintegration. But in most cases the degree of damage was such as to make accurate identification impossible.

† High background.

Cell suspensions. Table 1 shows the distribution of labelled cells in the lymph node suspensions, and Table 2 in suspensions of thymic cells. The problem of cell size in smears is always a very difficult one, since it depends upon a number of factors, including the nature of the medium in which the cells are suspended and the speed with which the smears are made. The slower the smear the greater the cell spread, and the apparent size of the cells. Absolute measurements are not therefore

of much value in smears, and in practice the relative size—i.e. the size in relation to other lymphocytes or to red blood cells—has proved the most useful guide.

The figures for size distribution in Tables 1 and 2 can only be regarded as an approximation in the middle part of the range. There is no doubt about the really large lymphocytes, or the small. But it is often difficult to decide whether a cell should be classified as a medium lymphocyte which is a little larger than usual, or a large lymphocyte which is rather smaller. The cytoplasm of the larger lymphocytes is generally more basophilic, and in doubtful cases this may be a useful additional criterion.

Table 2. *Number and size distribution of labelled cells in suspensions of thymus of guinea-pig at varying times after administration of tritiated thymidine*

No. of animal	Hr. after thymidine	No. of cells counted	Labelled cells, % of total	Size distribution of labelled cells				
				Large	Medium	Small	Damaged*	Total
100	0·5	500	12·6	14	18	2	29	63
101	1	500	9·6	2	5	1	40	48
94	3	900	10·5	4	24	2	65	95
88	4·75	1300	7·8	8	36	5	53	102
89	5	500	4·0	1	7	3	9	20
103	7	1100	14·2	13	50	29	64	156
106†	7	600	3·2	1	2	2	14	19
104	12	500	1·6	0	0	3	5	8
90	14·5	1000	10·6	12	21	36	37	106
105	17·5	500	2·0	0	0	8	2	10
92	27	1000	8·9	2	20	24	43	89
95	52·5	1000	11·7	1	12	46	58	117
91	78	1100	17·6	11	22	139	22	194
93	143	400	9·7	0	8	20	11	39
96	190·5	300	29·3	0	0	64	24	88

* Damaged; accurate indentification impossible.
† High background.

Subject to considerations such as these, the general trend seems clear. In the early stages there are hardly any labelled small lymphocytes, the bulk of the labelling cells being large and medium, whereas in the latter stages the number of labelled small lymphocytes gradually increases, while that of the large and medium cells steadily diminishes. This is in accord with the trend previously found in the cells of thoracic duct lymph (Everett, Reinhardt & Yoffey, 1960), and would appear to indicate that the cell content of the lymph reflects the changes in the parent lymphoid tissue. This orderly sequence of changes in size distribution of lymphocytes seems to fit in best with the view that as far as the lymphoid tissues are concerned, the small lymphocytes are the end stage of a series of cell divisions (cf. Sainte-Marie & Leblond, 1958).

The changes in intensity of labelling seem to fit in with this view. Though the actual grain counts have not been performed, the appearances strongly suggest that the grain count of the labelled cells steadily diminishes with the passage of time. This was equally the case both in lymph node and thymus, though it was our impression that there was a more rapid dilution of label in the thymus. In Expt. 96 (190·5 hr.) many of the labelled small lymphocytes had only one or two grains. There was some doubt at first as to whether one could in fact count these cells as

labelled or not, but in this instance there was fortunately a very clear background, which simplified the decision.

A further point of interest concerns the large number of damaged cells. Whilst many of these were undoubtedly reticulum cells, large numbers were also lymphocytes, and one could readily observe a graded series of damaged cells, ranging from those which could be clearly identified as lymphocytes which had been only slightly damaged, to cells which had been damaged so severely that if seen in isolation they would not have been recognizable. It seems apparent that many of these damaged cells, the 'smudge' or 'basket' cells thought so often to be effete and dying, are on the contrary cells which are actively growing (cf. Cronkite *et al.* 1959). It may possibly be the case that all growing cells, at certain phases of the mitotic cycle, are readily damaged in the process of making a smear.

DISCUSSION

The significance of the germinal centre

From appearances such as those seen in Pl. 1, fig. 2, it would appear that the germinal centre can be an area of active proliferation. Even when the centre is less active, as in Pl. 2, fig. 3, there still seems to be an appreciable amount of cell proliferation. The variation in activity of the different germinal centres seems to be brought out with especial clarity in the thymidine autographs, and would appear to accord best with the view that they are the seat of cyclic changes.

The evidence of DNA synthesis does not altogether rule out some degree of re-utilization, as suggested by Trowell (1957) and Hamilton (1957). However, if re-utilization does occur, it quite obviously does not dispense with the need for active formation of new DNA on a considerable scale. Similarly, the occurrence of energetic DNA synthesis does not rule out the possibility of some degree of re-circulation, as suggested by Yoffey & Drinker (1939). But the fact that new formation of DNA can be so marked as indicated in Pl. 1, fig. 2, seems to argue against anything like a major re-circulation of lymphocytes, as suggested by Sjövall (1936) and Gowans (1959). It is difficult to reconcile the evidence of thymidine uptake with the view of Latta (1922) that the germinal centre is primarily an area of degeneration, or that it is a reaction centre to noxious substances (Hellman, 1939), unless reaction implies active cellular proliferation presumably of cells other than lymphocytes. As far as Latta's (1922) view is concerned, it is perfectly true that in large germinal centres the central portion may show no mitotic activity, but in such cases it will usually be found that the peripheral part of the germ centre contains numerous dividing cells.

Lymphocyte formation in the spleen

The extent to which lymphocyte formation may occur in the spleen has always been a matter of doubt. Morris (1914) compared the white cell content of blood in the splenic artery with that in the vein, and found the count in the splenic vein to be appreciably above that in the artery. Pearce, Krumbhaar & Frazier (1918) were unable to demonstrate a clearcut difference, and concluded that '...detailed comparison of the arterial and venous blood of the spleen offers no evidence to indicate by the methods used that the spleen has an important role in blood formation'.

More recently, Fichtelius (1958) has suggested that the spleen may be constantly taking up lymphocytes from the blood stream. Appearances such as those in Pl. 2, fig. 5, do not rule out such a possibility, but they make it seem most unlikely. It seems improbable that a spleen which itself produces large numbers of lymphocytes would be in need of many lymphocytes from elsewhere. Furthermore, if the protective effect of spleen shielding (Jacobson, Marks, Gaston, Robson & Zirkle, 1949) is cellular in origin, this too would imply an active production of cells by the spleen.

SUMMARY

In thirteen healthy male guinea-pigs the uptake of tritiated thymidine has been investigated in lymph nodes, thymus, spleen and Peyer's patches. Radioautographs have been prepared both in cell suspensions, and in sections. The germinal centres frequently show extensive DNA synthesis, and this is interpreted to support the view that they are centres of cell proliferation. Lymphocytopoiesis is also active in Peyer's patches and in the spleen. In the lymphoid tissues small lymphocytes are formed by the repeated division of large cells.

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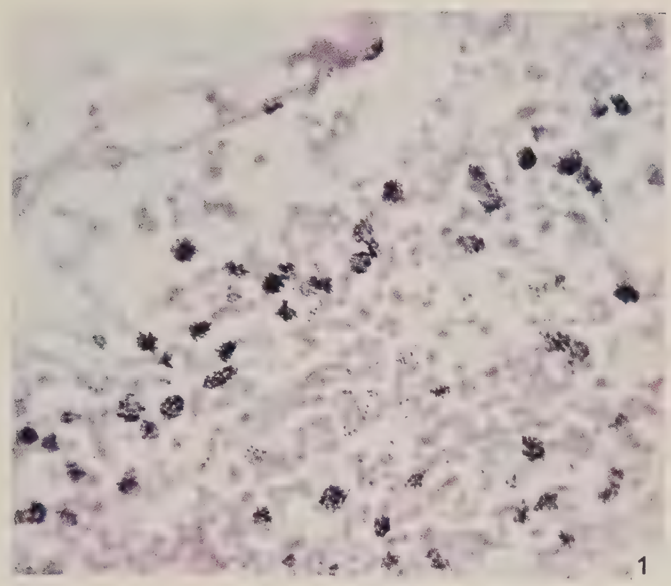

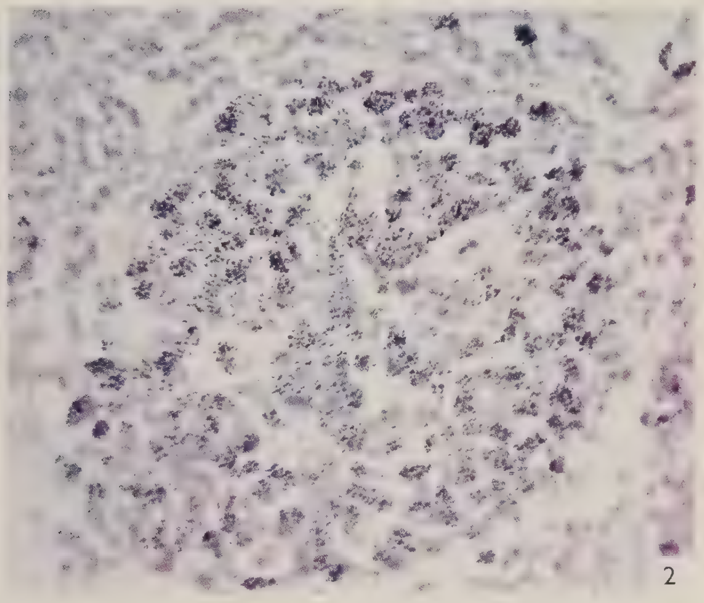

YOFFEY, REINHARDT AND EVERETT—UPTAKE OF TRITIUM-LABELLED THYMIDINE BY LYMPHOID TISSUE

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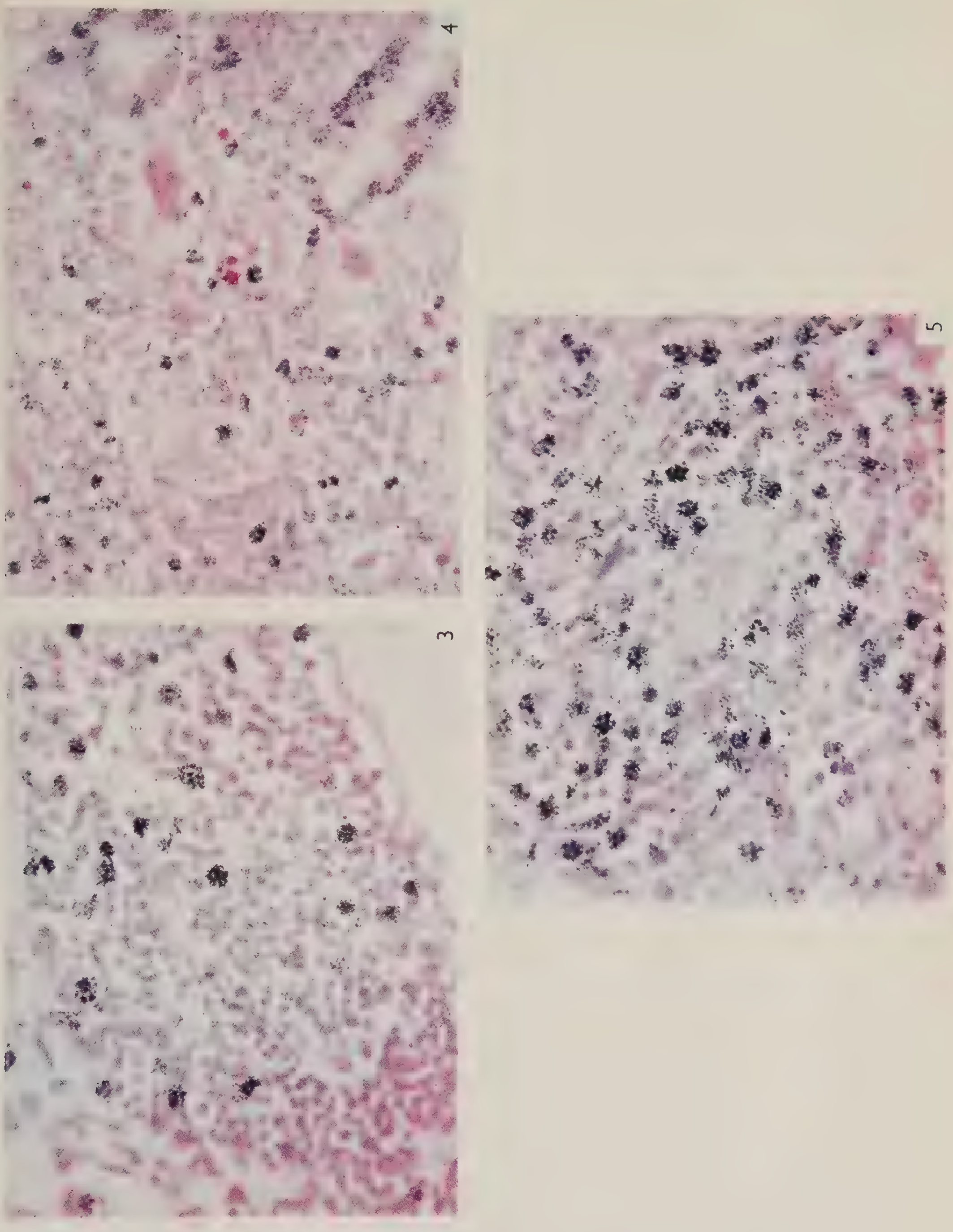

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Mesenteric lymph node, 4 hr. after tritiated thymidine. The section shows the periphery of a lymphoid nodule and the adjacent subcapsular region. A heavily labelled large lymphocyte is lying free in the subcapsular sinus. A considerable number of labelled large lymphocytes are lying in the lymphoid tissue close to the sinus. (×552.)

Fig. 2. Mesenteric lymph node of guinea-pig 4 hr. after tritiated thymidine. A young germ centre with many labelling cells. (×552.)

PLATE 2

Fig. 3. Mesenteric lymph node of guinea-pig. A lymphoid nodule with dense labelling of only a few cells. Compare with fig. 2. (×552.)

Fig. 4. Peyer's patch of guinea-pig, 4 hr. after tritiated thymidine. Note the heavy labelling of scattered cells in the patch, as also of the adjoining mucosal cells. (×368.)

Fig. 5. Spleen of guinea-pig 4 hr. after tritiated thymidine A perivascular lymphoid sheath showing many heavily labelled medium and large lymphocytes. (×552.)

THE FIBRINOID CAPSULE OF THE RAT PLACENTA AND THE DISAPPEARANCE OF THE DECIDUA

By D. BULMER AND A. D. DICKSON

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From the time of Duval's comprehensive work (1891) there have been several morphological investigations of the rat placenta. In some of these the descriptions have not been complete, nor have the interpretations placed upon the changes in the decidual cells always been justified. A more detailed account of some features, amplified where possible by histochemical findings, may help to clarify some of the problems involved.

MATERIAL AND METHOD

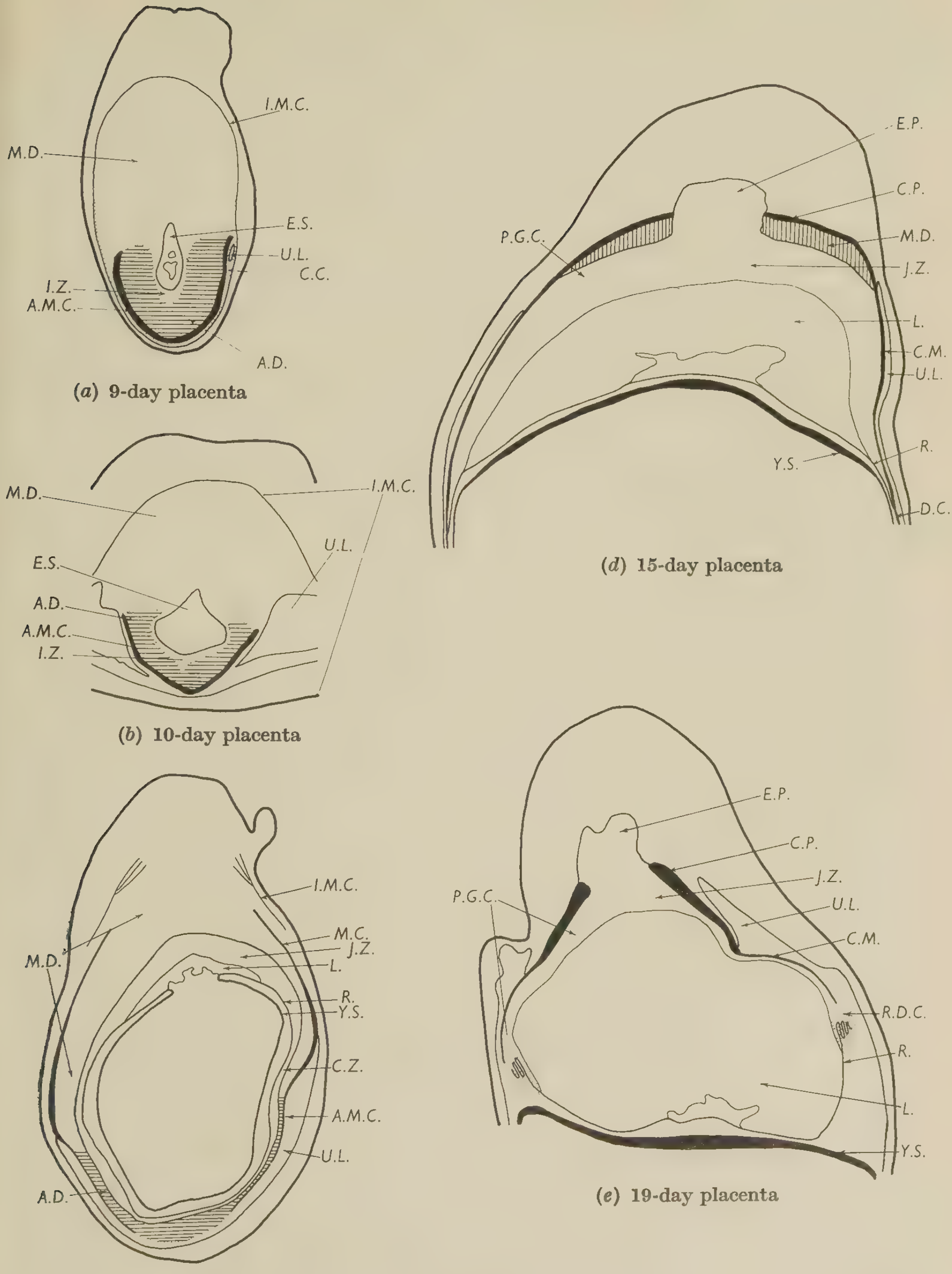
The material consisted of rat placentae of between 9 and 19 days, fixed in neutral formalin or acetic alcohol formalin for 24 hr. Series of sections, $5\,\mu$ thick, throughout the entire placental region were stained by the methods described in previous publications (Bulmer & Dickson, 1960; Dickson & Bulmer, 1960). In addition, sections were stained with Mallory's phosphotungstic acid-haematoxylin (Lillie, 1954) after mordanting with mercuric chloride (Peers, 1941), by the methyl green-pyronin technique (Pearse, 1960), by methyl green alone with the solution used by Alfert (1952), by the fast green method for nuclear histone (Alfert & Geschwind, 1953), by the chromalum-gallocyanin method (Pearse, 1960), with the azur B solution used by Flax & Himes (1952), by the acid solochrome cyanin technique (Pearse, 1957) and by the methods for bound lipid described by Berenbaum (1958). Tryptophan was demonstrated by the DMAB-nitrite method of Adams (1957) and reticulin fibres were impregnated by the techniques of Long (1948) and Gomori (Lillie, 1954).

OBSERVATIONS

The antimesometrial decidua

At the 9-day stage the antimesometrial decidua is clearly distinguished by the cytoplasmic basiphilia of almost all its cells, and is already less extensive than the more recently arisen mesometrial decidua (Text-fig. 1(*a*)). It bulges into the lumen of the proximal and distal inter-implantation regions and completely occludes the lumen in the implantation region.

Text-fig. 1. Diagrammatic sections of 9- to 19-day rat placentae. (*a*), (*c*), (*d*) and (*e*) are at right angles to the long axis of the uterus, while (*b*) is parallel to the long axis. *I.M.C.* inner muscle coat, *M.D.* mesometrial decidua, *E.S.* embryonic sac, *A.M.C.* antimesometrial capsule, *A.D.* antimesometrial decidua, *I.Z.* implantation zone, *U.L.* uterine lumen, *C.C.* cellular condensation in the plane of the uterine lumen, *M.C.* mesometrial capsule, *J.Z.* junctional zone, *L.* labyrinth, *R.* Reichert's membrane, *Y.S.* yolk sac, *C.Z.* central zone, *C.P.* paracentral capsule, *C.M.* marginal capsule, *P.G.C.* placental giant cells, *D.C.* decidua capsularis in apposition with Reichert's membrane, *E.P.* endovascular plasmodium, *R.D.C.* remnant of decidua capsularis at placental margin.



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Various cell types may be distinguished. In the implantation zone (Krebheil, 1937), around the antimesometrial pole of the embryonic sac and extending mesometrially as a thin layer on either side of the sac, degenerating decidual cells lie in a mesh of vascular spaces with the foetal giant cells of the central zone (Everett, 1935) or Durchdringungszone (Grosser, 1927) immediately internal to them. The decidual nuclei here are of irregular shape, some small with their chromatin clumped in relatively large granules and some fragmented. In sections stained with a basic thiazine dye this zone is clearly distinguished by the absence of cytoplasmic basiphilia (Pl. 1, fig. 1).

Elsewhere the antimesometrial decidua is formed principally of large cells, often binucleate and with the nuclei large and open-faced. These cells are similar to those which occur in the corresponding situation in deciduomata, and which Sachs & Shelesnyak (1955) considered to be polyploid. The cytoplasm is markedly basiphilic, more so in some cells than in others and generally more in the inner layers, just outside the implantation zone, than in the outer layers. Mitoses are restricted to the more peripheral layers. Among the decidual cells is an extensive network of small vessels, lined by large endothelial cells with densely staining cytoplasm and compact nuclei. Limiting the antimesometrial decidua externally is the layer of condensed tissue which may be termed the capsule (Bulmer & Dickson, 1960). This stains a deep brown colour in trichrome preparations and with phosphotungstic acid-haematoxylin there are irregular patches of deep-blue staining. It contains numerous nuclei, smaller than those of the decidual cells and larger than those of the surrounding stroma, but along its inner margin nuclei can be identified which are intermediate in appearance between the decidual and capsular nuclei (Pl. 1, fig. 2). This antimesometrial capsule was noted by Grosser (1927) as a layer of degenerating connecting tissue and by Holmes & Davies (1948) as a layer of flattened decidual cells. A similar condensed band was observed in deciduomata by Velardo, Dawson, Olsen & Hisaw (1953). Immediately external to the capsule is a fairly dense condensation of stromal cells which indicates the plane of, and is in part split by, the new uterine lumen (Text-fig. 1(*a*)). Outside this is the inner muscle coat of the uterus.

At the 10-day stage the antimesometrial decidua still forms a thick layer (Text-fig. 1(*b*)). The capsule lies inside the stromal zone of the uterine lumen and now stains intensely with the picric acid differentiator in trichrome preparations. Many of the antimesometrial decidual cells have a yellow-staining cytoplasm, others stain a deep brown colour, while there is a discontinuous layer of large cells, just internal to the capsule, with cytoplasm which is unstained in trichrome preparations (Pl. 1, fig. 3). It is notable that these latter cells are the only antimesometrial decidual cells to show mitotic figures at this stage.

Despite the absence of basic staining with haematoxylin the capsular matrix shows a marked basiphilia with azur A and pyronin (Pl. 1, fig. 4). This is abolished by previous extraction for 15 min. at 90° C. with 5% trichloracetic acid, but unlike the cytoplasmic basiphilia of the antimesometrial decidual cells is not markedly reduced by a prior digestion with ribonuclease (Pl. 1, fig. 5). While the capsule is moderately PAS-positive, stains faintly with Gomori's aldehyde fuchsin and red in acid solochrome cyanin preparations, it stains orthochromatically with azur A and

does not stain with alcian blue. It does, however, stain metachromatically with the azur B solution of Flax & Himes (1952) and binds chromalum-gallocyanin at pH 1·64. It is possible that the basiphilia of the capsule, therefore, is due to the presence of ribonucleic acid in a form more resistant to nuclease digestion after formalin fixation than the ribonucleic acid of the decidual cells. The capsule stains strongly with the coupled tetrazonium technique, moderately with the methods for bound lipid described by Berenbaum (1958) and shows a strong reaction with the DMAB-nitrite method, of similar intensity to that of fibrin. With phosphotungstic acid-haematoxylin it gives a deep blue colour, and the cytoplasm of many of the antimesometrial decidual cells now reacts similarly with this technique. Silver impregnation methods for reticulin demonstrate sparse argyrophil fibres in the capsule and outline the walls of the numerous small vessels within it.

The capsular nuclei stain only very faintly with haematoxylin in trichrome preparations, often with a yellowish tinge, and show a reddish colour with acid solochrome cyanin. Many are of irregular shape and some appear fragmented. They are strongly basiphilic with azur A, Feulgen-positive and methyl green-positive and stain well with the fast green method for nuclear histone (Pl. 1, fig. 6). While the nuclei of the internal layers of the antimesometrial decidua, including those of the implantation zone, stain strongly with the fast green method, staining is much fainter in the outer layers, particularly in the nuclei of the large cells which form a discontinuous layer just within the capsule.

At the 12-day stage most of the antimesometrial decidua forms a thin decidua capsularis, though the penetration of the new uterine lumen is not yet complete (Text-fig. 1 (*c*)). An outer condensed layer, with staining reactions similar to those of the capsule at the 10-day stage, is present laterally and now extends mesometrially outside the marginal portion of the mesometrial decidua. It is difficult to define precisely where the junction lies between antimesometrial decidua and mesometrial decidua, but there is no doubt that the capsule and, to a lesser extent, the uterine lumen, have now come into relation with the periphery of the mesometrial decidua. The mesometrial decidua, therefore, as well as the antimesometrial decidua, contributes to the decidua capsularis.

Around the antimesometrial pole the decidua capsularis consists of up to about a dozen layers of degenerating cells (Pl. 2, fig. 7). The nuclei are irregularly shaped or fragmented, but stain strongly with the fast green method and are Feulgen- and methyl green-positive. They are contained in a matrix which gives a moderate diastase-fast PAS reaction and, in contrast to the earlier stages, shows occasional granules of glycogen. Phosphotungstic acid-haematoxylin produces an orange colour, with patches of intense blue staining, and there are extensive accumulations of basiphilic material which can be removed by ribonuclease digestion. In the regions around the antimesometrial pole which are not penetrated by the uterine lumen a thin capsular band separates the degenerating decidua from the stromal zone. Where the uterine lumen is present, however, the capsule is usually deficient and the degenerating tissue is exposed to the lumen. Laterally, where the capsule forms a well-defined band, a few layers of degenerating cells may lie inside it, but the whole decidua capsularis is very much thinner here than in the region of the antimesometrial pole, and sometimes consists of the capsule alone. Near the margin of the

chorio-allantoic placenta the degenerating cells are replaced by healthy cells, similar in structure to those of the decidua basalis. Internally, the decidua capsularis lies adjacent to the rich vascular bed of the central zone. Many of the foetal giant cells of this zone are now beginning to degenerate, with flattening, elongation and vacuolation of the nuclei—a process similar to that occurring at a rather later stage in the placental giant cells (Dickson & Bulmer, 1960). There are accumulations of leucocytes in the central zone, in the decidua capsularis and in the uterine lumen.

At the 13-day stage degeneration of the antimesometrial decidua is almost complete and the capsule is mainly restricted to the mesometrial decidua. Where it is related to the uterine lumen its outer surface is often covered with a low cubical epithelium (Pl. 2, fig. 8). Antimesometrially, between Reichert's membrane and the uterine epithelium and among the maternal vessels of the central zone, are a few layers of giant cells, most of them degenerate, with a few small nuclear remnants of decidual cells and leucocytes external to them.

At the 15-day stage (Text-fig. 1(*d*)), only a very thin layer separates Reichert's membrane from the uterine epithelium antimesometrially. Nuclear remnants of decidual cells, giant cells or leucocytes are very rare, except near the margin of the chorio-allantoic placenta (*v. infra*).

The mesometrial capsule

It has been mentioned that at the 12-day stage the capsular layer of the antimesometrial decidua extends mesometrially. It forms a thin band outside the marginal part of the decidua basalis (Pl. 2, fig. 9), and immediately external to it there is a thin layer of stroma, containing occasional metrial gland cells, which separates it from the inner muscle coat of the uterus. The capsule is deficient over a large area across the base of the mesometrium (Text-fig. 1(*c*)), where the decidua is separated from the mesometrial triangle only by the fibres of the inner muscle coat, intermingled with numerous metrial gland cells. The mesometrial capsule resembles the antimesometrial capsule in its appearance and staining reactions. It binds the picric acid of trichrome preparations, and the intense orthochromatic basiphilia with azur A is resistant to ribonuclease digestion. It contains small, dense nuclei which stain strongly with the Feulgen, methyl green and fast green methods, but faintly and with a yellowish tinge in trichrome preparations. The capsular matrix stains intensely blue with phosphotungstic acid-haematoxylin, there is faint staining with aldehyde fuchsin and alcian blue, and a strong reaction with the DMAB-nitrite technique.

Text-fig. 1(*d*) will serve to illustrate the main features of the 14- and 15-day stages. The capsule is a well-marked structure, considerably thicker than at 12 days. It extends further across the base of the mesometrium, but does not form a complete investment for the decidua basalis. The aperture remaining at the mesometrial pole is traversed by the endovascular plasmodium lining the maternal arteries of the placenta (Dickson & Bulmer, 1961). At the margin of the chorio-allantoic placenta the capsule is continuous with the decidua capsularis, which here consists of an amorphous mass with a few small nuclear remnants lying outside an interrupted

layer of central zone giant cells, many of which are degenerating. This mass is continuous with the very narrow remnant of the antimesometrial part of the decidua capsularis, which has been noted above.

The uterine lumen extends mesometrially around the marginal part of the chorio-allantoic placenta, and here the 'marginal' part of the capsule forms a thin layer internal to the lumen (Pl. 2, fig. 10). In some regions a low cubical or squamous epithelium clothes its outer surface (Duval, 1891). Immediately adjacent to it internally are the foetal giant cells, and the layer of decidua which separated this portion of the capsule from the giant cells at the 12-day stage has either disappeared or been incorporated into the capsule. Though the marginal part of the capsule is rather thicker, presumably because of the incorporation of more tissue in its inner layers, it closely resembles the mesometrial capsule of the 12-day placenta in appearance, staining reactions and nuclear content.

Beyond the mesometrial limit of the uterine lumen, between it and the central aperture of the capsule, a thin layer of decidua separates the 'paracentral' part of the capsule from the foetal giant cells. In contrast to the marginal portion, the paracentral portion of the capsule shows little sign of degeneration. It is traversed by vascular channels, and is distinguished from the stroma outside and the decidua inside by its deeper brown staining in trichrome preparations (Pl. 2, fig. 11). The nuclei are of regular shape, closely packed and stain well. The matrix is basiphilic, though less markedly so than that in the marginal portion, and the basiphilia is more readily removable with ribonuclease digestion.

It can be seen that the uterine lumen and the capsule extend further mesometrially around the chorio-allantoic placenta at this stage, though this may be due in part to antimesometrial growth of the placenta itself. The size of the central aperture in the capsule is actually less at the 14-day than at the 12-day stage, and the paracentral portion of the capsule appears to be a new formation. In the 12-day placenta, however, the decidual cells in the position occupied by the paracentral portion of the capsule at the 14-day stage are smaller than those lying internal to them, and are flattened along a line continuous with the capsule (Pl. 2, fig. 12). It is likely that the paracentral portion of the capsule arises from these cells.

With further development the capsule retains its position. Degenerative changes, which at the 12- to 15-day stages are confined to the marginal portion, also occur in the inner layers of the paracentral portion. The layer of decidua basalis between the paracentral portion and the foetal giant cells becomes reduced, and the cytoplasm of its cells stains a deep blue colour with phosphotungstic acid-haematoxylin. By the 17-day stage this decidual layer has effectively disappeared (Dickson & Bulmer, 1960). In some areas, either immediately adjacent to the capsule or incorporated in its inner layers, are masses of closely packed degenerate nuclei, apparently remnants of decidual cells. Elsewhere the capsule is in immediate contact with the foetal giant cells, many of which are degenerate and incorporated within its substance (Pl. 2, fig. 13).

At the 19-day stage (Text-fig. 1(*e*)) the uterine lumen has extended still further mesometrially, so that the placenta is attached by a relatively narrow pedicle. The marginal portion of the capsule incorporates many degenerate giant cells as well as decidual nuclei. Paracentrally, the capsule is very much thicker. Though

degenerative changes, with clumps of nuclear remnants, occur in the inner layers, the outer layers consist of closely packed and deeply staining cells (Pl. 2, fig. 14). The central aperture of the capsule is still evident, though the outgrowth of endovascular plasmodium is now degenerating. In addition to the thickening of the paracentral part of the capsule, there is an extensive proliferation of small, deeply staining stromal cells at the base of the mesometrium, in the region of the metrial gland.

One further point may be mentioned. The mesometrial capsule lies between the decidua basalis and the stromal zone which is eventually largely split by the uterine lumen. In two of our specimens, one at the 15-day and one at the 17-day stage, there is a further band of condensed tissue at the periphery of this stromal zone, outside, and roughly parallel to, the paracentral part of the capsule. This band, which from its staining reactions appears to be collagenous, does not cross the mesometrial triangle, but can be followed antimesometrially into continuity with a dense connective tissue layer immediately beneath the uterine epithelium.

DISCUSSION

The capsule which we describe, first appearing in relation to the antimesometrial decidua and persisting as a feature of the mesometrial decidua, has not been reported in detail by previous workers. Bridgman (1948) described a dense connective tissue layer around the entire decidua from the 9th day onwards, and the subsequent splitting of this layer by the extension of the new uterine lumen. It is difficult to reconcile our findings with Bridgman's account. The dense connective tissue layer she described may correspond with the stromal zone which forms the plane of extension for the new lumen. This is a fairly dense, cellular condensation at the 9-day stage, though it then contains no demonstrable collagen fibres and is deficient mesometrially. In the later stages the mesometrial portion of this zone, beyond the extending uterine lumen, is a very loose tissue, and it is difficult to believe that this could be the structure which Bridgman described as a dense connective tissue layer, separating the decidua basalis from the myometrium. It would appear that Bridgman must have been referring to the mesometrial portion of the capsule which we describe. If this is so, her account of a dense connective tissue layer seems misleading, and her identification of the capsule as the plane of the uterine lumen inaccurate. The mesometrial portion of the capsule in the later stages of gestation was noticed by Holmes & Davies (1948), who described the compression of the decidua basalis into a narrow band, except for a persistent tuft around the central artery. Despite the description by Duval (1891) of the disappearance of the decidua basalis, he made no mention of the mesometrial part of the capsule.

The capsule of the antimesometrial decidua may originate in part from the layer of stromal cells which lies external to it, but the morphological appearances of the nuclei, particularly at the 9-day stage, suggest that decidual cells are incorporated into its internal layers. In the mesometrial decidua, the appearances at the 15-, 17- and 19-day stages indicate that degenerate decidual cells and giant cells are incorporated into the inner layers of the capsule, while the outer layers are formed by proliferation of stromal cells.

Grosser (1927) and Bridgman associated the function of the capsule with the subsequent formation of the uterine lumen. The plane of the capsule, however, is not the plane of extension of the lumen, though obviously the formation of the capsule may facilitate the spread of the lumen outside it. The degeneration in the inner layers of the paracentral part of the capsule by the 19-day stage may provide a plane for placental separation, and the proliferation of stromal cells in its outer layers and in the mesometrial triangle may be associated with the post-partum regeneration of the endometrium.

The nature of the capsular material presents a difficult problem. Its staining reactions may justify the term fibrinoid, though there are obvious differences, particularly in basiphilia, from those of fibrin. The intense basiphilia of the anti-mesometrial capsule and of the degenerate part of the mesometrial capsule may be due to an accumulation of ribonucleic acid, presumably derived from the degenerate cells which they incorporate. The staining with gallocyanin at low pH suggests the presence of either nucleic acid or acid mucopolysaccharide. The lack of appreciable staining with alcian blue, the orthochromasia with azur A and the removal of the basiphilia with hot trichloracetic acid might be taken to exclude the presence of acid mucopolysaccharide (Swift, 1955). On the other hand, there is a very marked resistance to nuclease digestion, and nucleic acid would be expected to stain blue with acid solochrome cyanin (Pearse, 1957). The lack of basic staining with alum haematoxylin in trichrome preparations is an interesting feature, and the staining with picric acid, both in the capsular material and in the nuclei, suggests the accumulation of a basic protein which, like the protein of red blood cells, binds picric acid in preference to the plasma stain. The degeneration process must be associated with the freeing of a large number of stainable groups, both basic and acidic, and also with the presence of a relatively high tryptophan content.

Degenerative changes and fragmentation occur in the nuclei of the antimesometrial capsule from the 10-day stage onwards, and in the nuclei of the marginal portion of the mesometrial capsule from the 12-day stage. The degenerating nuclei retain their affinity for methyl green, though recent opinion (Alfert, 1952; Rosenkranz & Bendich, 1958) indicates that methyl green stainability is not, as Kurnick (1950) suggested, a simple index of the degree of polymerization of desoxyribonucleic acid. The significance of the staining differences in the antimesometrial decidua demonstrated with the fast green method is obscure. The rat placenta, with the occurrence of polyploidy (Sachs & Shelesnyak, 1955) followed by degenerative changes in decidual cells and the appearance and subsequent degeneration of polyploid giant cells would seem to offer a fertile field for microspectrophotometric studies.

The degeneration and disappearance of both antimesometrial and mesometrial decidua is a striking feature of the rat placenta. While appearances suggest that the external layers of each are incorporated into the capsule, other factors must also be involved. The degeneration of the decidua capsularis may be due, as Young (1956) suggested, to an impairment of its vascular supply, though Everett (1935) found that the vessels of the central zone contain actively circulating blood at the 13- to 14-day stage. The work of Velardo *et al.* (1953) on deciduomata, where there is a similar degeneration of the antimesometrial decidua with the appearance of a

condensed zone surrounding it, implies that capsule formation and decidual degeneration are not dependent upon the presence of an expanding embryonic sac. Sachs & Shelesnyak suggested that the abnormal reproductive mechanism associated with the appearance of polyploidy in deciduomata may be responsible for the subsequent degeneration, and such an explanation could also be applicable to the decidual degeneration of pregnancy.

Phagocytosis by the foetal giant cells has been held to be largely responsible for the removal of the degenerate decidual tissue (Bridgman). Morphological evidence that this is an important mechanism in the removal of the decidua capsularis is doubtful and unconvincing, and we have recorded a similar opinion on the removal of the decidua basalis (Dickson & Bulmer, 1960). The leucocytosis, which occurs in both the decidua capsularis and decidua basalis, may be involved in the removal of the degenerate tissue. There may be autolysis of the decidua, as Duval suggested for the decidua capsularis, and absorption of the products of autolysis by the large vessels of the decidua basalis, the vessels of the central zone or the uterine epithelium.

SUMMARY

1. A capsule appears by the 9-day stage, limiting the periphery of the antimesometrial decidua. The basiphil cells of the antimesometrial decidua degenerate, and by the 15-day stage the decidua capsularis has effectively disappeared.

2. At the 12-day stage the capsule extends around the mesometrial decidua, though it never reaches the mesometrial pole. The mesometrial decidua gradually disappears, in part by incorporation into the capsule.

3. The histochemical features of the capsule and of the degenerating decidual tissue are described and discussed. The capsular matrix is probably justifiably termed fibrinoid.

4. There is no convincing morphological evidence that the foetal giant cells phagocytose degenerate decidual tissue.

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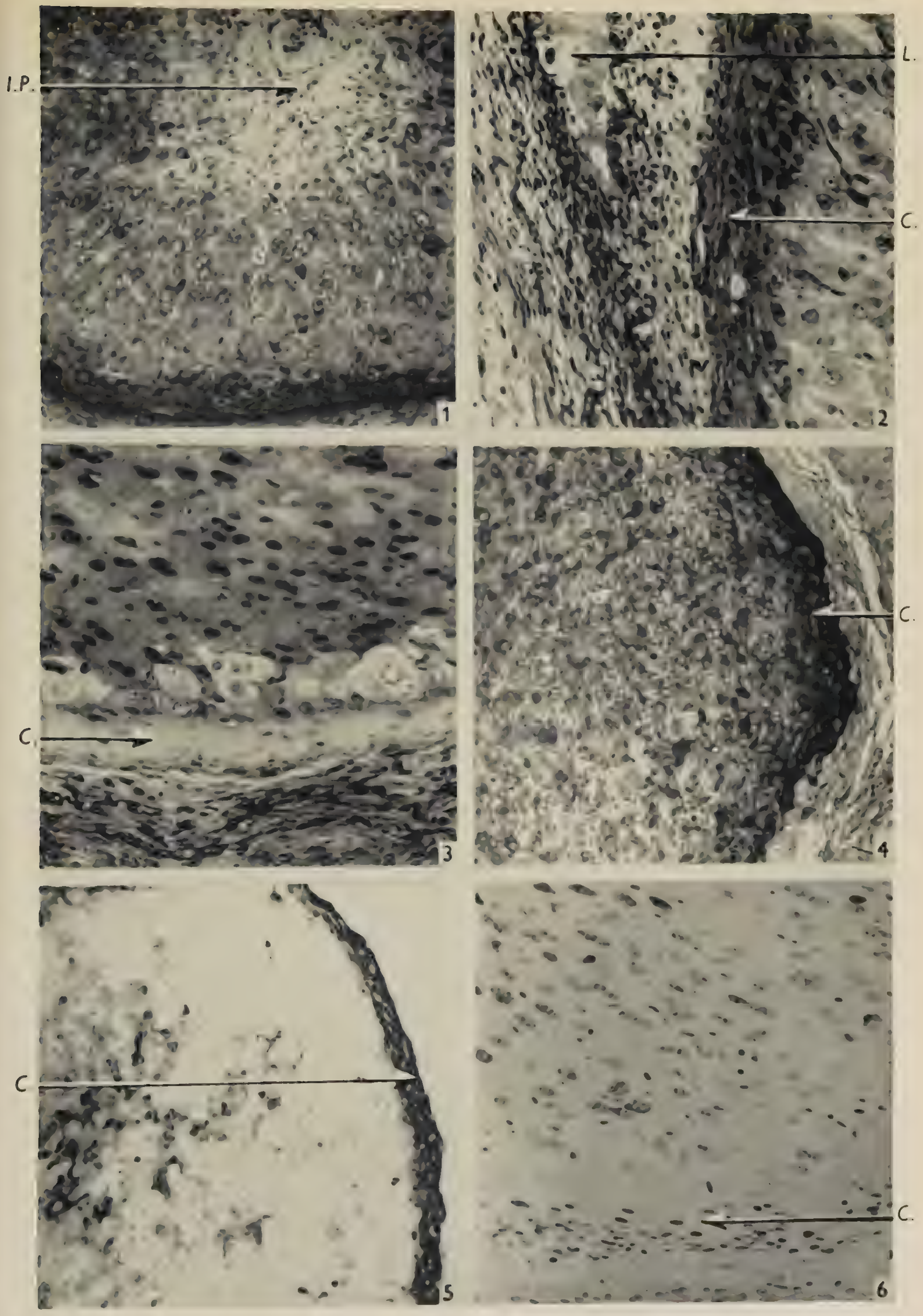
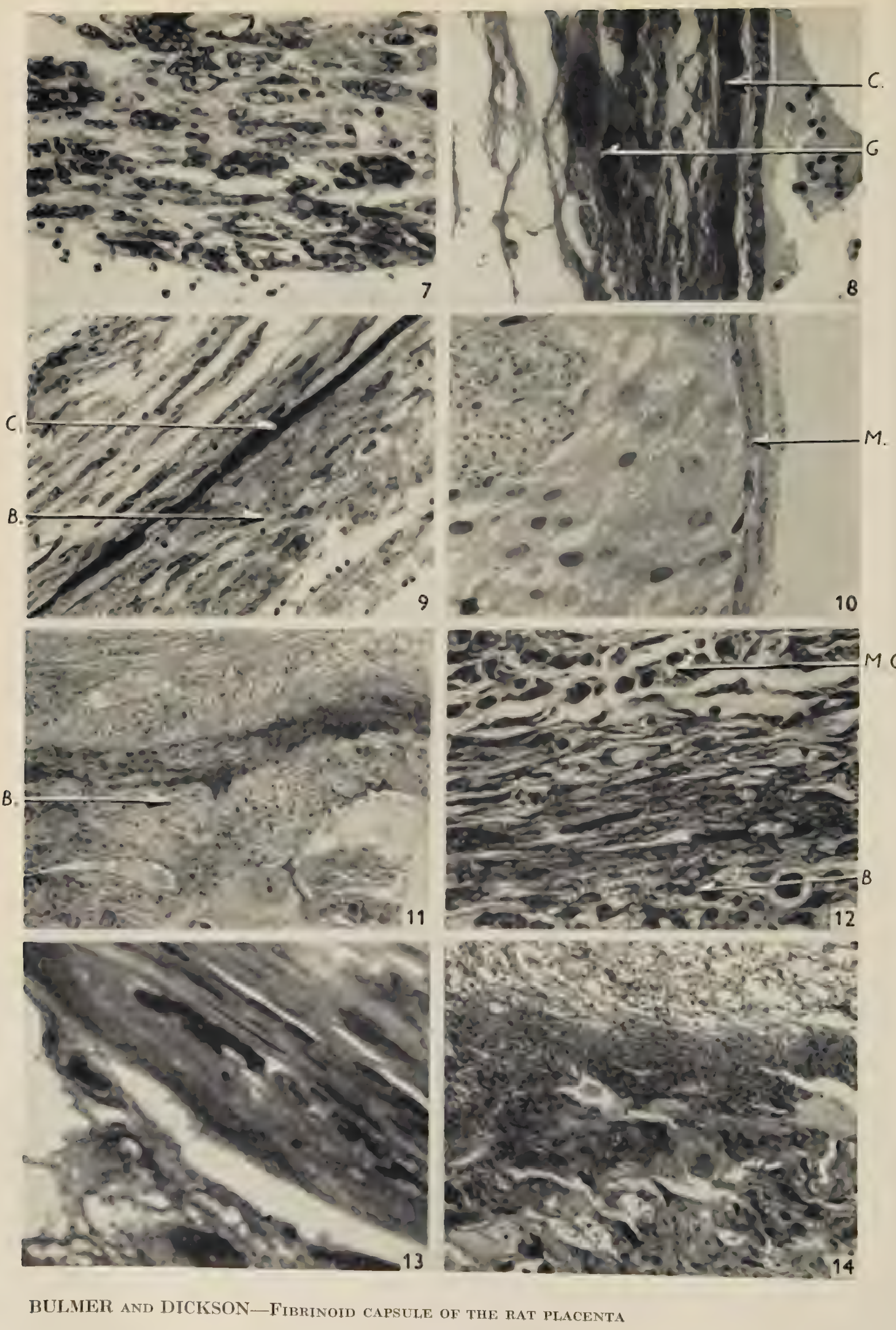


BULMER AND DICKSON—FIBRINOID CAPSULE OF THE RAT PLACENTA

(*Facing p.* 308)



BULMER AND DICKSON—FIBRINOID CAPSULE OF THE RAT PLACENTA

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Antimesometrial decidua of a 9-day placenta. The implantation zone is shown at *I.P.* Azur A, ×75.

Fig. 2. Periphery of the antimesometrial decidua of a 9-day placenta. *C.* indicates the capsule and *L.* the uterine lumen. Trichrome, ×200.

Fig. 3. Periphery of antimesometrial decidua of a 10-day placenta, showing large subcapsular cells with unstained cytoplasm. Trichrome, ×200.

Fig. 4. Capsule and antimesometrial decidua of a 10-day placenta. Azur A, ×75.

Fig. 5. A similar section to that shown in Fig. 4, but which has been subjected to ribonuclease digestion. Azur A, ×75.

Fig. 6. Nuclei of antimesometrial decidua and capsule stained by alkaline fast green method. *D*, antimesometrial decidua. ×200.

Plate 2

Fig. 7. Degenerate decidua capsularis in the region of the antimesometrial pole at the 12-day stage. Methylene blue, ×250.

Fig. 8. Decidua capsularis close to margin of chorio-allantoic placenta at the 13-day stage. The surface of the capsule is covered by a low cubical epithelium. Central zone giant cells are indicated at *G.* Trichrome, ×200.

Fig. 9. The mesometrial capsule at the 12-day stage, outside the decidua basalis, *B*. Methylene blue, ×200.

Fig. 10. The marginal portion of the capsule (*M.*) at the 14-day stage. Trichrome, ×75.

Fig. 11. The paracentral portion of the capsule at the 14-day stage. Trichrome, ×75.

Fig. 12. The paracentral area at the 12-day stage. The flattened cells between the decidua basalis and the region of the metrial gland (*M.G.*) indicate the future paracentral part of the capsule. P.T.A.H., ×400.

Fig. 13. The paracentral portion of the capsule at the 17-day stage. The decidua basalis in this region has disappeared, and a degenerate giant cell nucleus is incorporated into the capsule. Trichrome, ×200.

Fig. 14. The paracentral portion of the capsule at the 19-day stage, showing the proliferation of stromal cells in its outer layers. Trichrome, ×75.

THE SUBPLACENTA OF THE GUINEA PIG: AN ELECTRON MICROSCOPIC STUDY

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The development, histology and histochemistry of the guinea-pig subplacenta have been described in a previous paper in this *Journal* (Davies, Dempsey & Amoroso 1961). The subplacenta is a specialized chorionic zone derived by proliferation and infolding of the foetal ectoderm in the floor of the 'central excavation' or mesodermal core of the chorio-allantoic placenta. It consists of cytotrophoblastic lamellae enclosing chorio-allantoic mesenchyme on their foetal surface and giving rise to syncytial trophoblast on their maternal surface. The syncytium of the subplacenta differs from that of the chorio-allantoic placenta in that it is vascularized exclusively by foetal vessels and contains, instead of maternal blood channels, a system of lacunar spaces filled with amorphous protein-like material. This material gives a strong periodic acid-Schiff (PAS) reaction after the removal of glycogen by saliva and is probably glycoprotein in nature. In addition, the syncytial cytoplasm shows a delicate PAS-positive stippling, indicating the presence of granules or droplets just within the resolving power of the light microscope, and also contains a large amount of glycogen. The subplacental syncytium is prolonged into the basal decidua in the walls of the maternal placental vessels, the endothelium of these vessels being replaced by trophoblastic cells at an early stage. Due to some doubt concerning the cytotrophoblastic or syncytial nature of the vessel wall in such areas the term 'endotrophoblast' was used. Arguments were put forward for a possible gonadotrophic function of the subplacenta or, alternatively, for its possible involvement in the absorption of substances of high molecular weight from the decidua and their transport to the foetal circulation.

The electron-microscopic observations in this paper have clarified many of the points in the previous paper which could not be answered owing to the inherent limitations of the light microscope. Most especially they have revealed the true nature of the lacunar spaces and the relationship of these to the intercellular spaces of cytotrophoblastic layer, as well as the fine structure of the syncytial trophoblast and its 'endotrophoblastic' extensions into the walls of the decidual vessels.

MATERIAL AND METHODS

Specimens were obtained from pregnant guinea-pigs at various stages of gestation from about the 20th day to term (68 days). Portions of the placenta were fixed by immersion in the osmium tetroxide mixtures recommended either by Palade (1952) or Dalton & Felix (1955). Adequate fixation was obtained after 1 hr. Tissues were

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then dehydrated and embedded in a mixture of methyl- and butyl-methacrylate in a ratio of 1:6 or 1:8. Sections were cut on a Porter–Blum microtome and, after orientation by the use of the phase-contrast microscope, were examined in the electron microscope (RCA model EMU 2E).

RESULTS

The following observations with the electron microscope cover a wide range of placental areas in the guinea-pig placenta, viz, the subplacenta, the 'coarse' and 'fine' syncytium of the chorio-allantoic labyrinth, the parietal endoderm and its underlying layer of chorionic giant cells, the modified walls of the major placental vessels at various levels, and the decidual cells. The interrelationship of these parts has been illustrated diagrammatically in a previous paper (Davies, *et al.* 1961). Many of these observations are of a limited nature, the subplacenta and vessel walls alone being considered in detail. However, a survey of such a wide range of placental structures has been justified on the grounds that they form an integrated whole in terms of development and probably also of ultimate physiological function.

Terminological note

The term 'endoplasmic reticulum' is used to describe the vesicular and membranous structures of indeterminate origin within the cytoplasm. The term 'ergastoplasm' is used only when these membranous and vesicular elements of the endoplasmic reticulum are associated with small granules (Palade, 1952). Such cytoplasmic areas are generally held to show marked cytoplasmic basophilia after staining with the basic aniline dyes.

Subplacenta

The subplacenta at the 25th day is illustrated as seen in the phase-contrast microscope (Pl. 1, fig. 1) and at a low magnification in the electron microscope (Pl. 2, fig. 2). The cytotrophoblastic layer consists of a two or three-layered epithelium resting on a thin basement membrane which separates it from the foetal mesenchyme. There are indications that the epithelium is pseudo-stratified and that all the cytotrophoblastic cells may reach the basement membrane at some point. The adjacent walls of the cells are moulded by mutual pressures. For the most part the walls are in contact, the apposing cell membranes being modified in some areas by the development of terminal bars. Between the areas of apposition the intercellular spaces are irregularly dilated, especially at the angles between two or more cells. The presence of minute protrusions of the cell membrane or microvilli into the spaces suggests that they are not shrinkage artifacts but that they may exist in the living state. The nuclei of the cytotrophoblastic cells are large relative to the volume of the cytoplasm and contain several prominent skein-like nucleoli. The cytoplasm is finely granular and contains a few mitochondria but no fat or other inclusions. These cells, which show marked cytoplasmic basophilia in the light microscope, contain many of the dense particles which have been correlated with such basophilia in other sites (see Palade, 1952). The syncytial trophoblast, which is formed by proliferation from the cytotrophoblast, is limited externally by a definite cell membrane where it faces the cytotrophoblastic layer. At the interface between

syncytial and cytotrophoblastic elements of the subplacenta, several situations may exist. The plasma membranes of the two layers may lie in simple apposition, being thickened in some areas by the development of terminal bars. In other places the two layers are separated producing an extracellular cleft lined by microvilli which is continuous with the dilated intercellular spaces of the cytotrophoblastic layer. The cytoplasm of the syncytial trophoblast is coarsely granular and contains a few mitochondria which are smaller than those of the cytotrophoblastic cells and are also less dilated. Patches of flocculent material probably represent deposits of glycogen. The large vacuoles or lacunae observed in the subplacental syncytium (Pl. 1, fig. 1) appear in the electron microscope as irregular spaces lined by microvilli and occupied by a small amount of coagulated material. In the early stages of pregnancy (Pl. 2, fig. 2) many of the microvilli are swollen, suggesting a hydropic change, perhaps associated with an active transfer of water into or out of the lacunae.

The changes in the subplacenta with advancing gestation are shown in Pl. 3, fig. 3. The cytotrophoblastic layer is reduced to a single row of cells though there are many regions where this layer remains multilaminar until at least the 50th day. The cytotrophoblast is absent in some areas allowing the syncytium to rest directly on the basement membrane. In these areas there is a clearly defined space between the syntium and the basement membrane. Delicate microvilli, protruding from the plasma membrane of the syncytium, project into the space and some are implanted on the basement membrane by small, foot-like processes. Elsewhere, along the boundary between the syncytium and the cytotrophoblastic layer, the syncytium is less intimately applied to the cellular layer than was the case in the earlier stages and is separated from it by a cleft-like space into which project microvilli derived from the plasma membrane of the syncytium. The true syncytial character of the subplacental syncytium is also shown in Pl. 3, fig. 3. The lacunar spaces are lined by microvilli similar to those at the interface between the syncytium and the cytotrophoblastic layer.

Pl. 4, fig. 4, illustrates the details of the subplacental syncytium at the middle of gestation. The syncytial mass is traversed by a system of vacuoles or lacunae. Many of the lacunae are dilated and lined by delicate microvilli, the contained material being sparse and of moderate electron density. In other areas the lacunae are narrowed or partially collapsed. In these the microvilli are absent or poorly developed and the contained material is of higher electron density. It is reasonable to suppose that the occurrence of the most electron dense material in association with an absence or reduction in the number of microvilli indicates stagnation of the lacunar contents. The syncytial cytoplasm contains a few scattered mitochondria. There are large areas of the cytoplasm occupied by featureless masses of granular material of low electron density, probably representing glycogen in which the subplacental syncytium is particularly rich (Davies, *et al.* 1961). Isolated islands of ergastoplasm are also found. The ergastoplasmic membranes are arranged as parallel tubules which contain amorphous material resembling in over-all electron density the material within the syncytial lacunae. The syncytial cytoplasm also contains many granules or droplets of marked electron density. These droplets are just within the limits of resolution of the light microscope and may correspond with the stippled areas of PAS positive material previously described in the subplacental

syncytium (Davies, *et al.* 1961). They show no predilection for any part of the cytoplasm, being found as often in the vicinity of the nucleus as in the neighbourhood of a lacunar space.

The junction between the subplacental syncytium and the cytotrophoblast is illustrated at higher magnification in Pl. 5, fig. 5. The plasma membrane of the syncytial trophoblast is in smooth apposition with the surface of the cytotrophoblastic cells but shows localized dilations lined by microvilli and continuous with the intercellular spaces of the cytotrophoblastic layer. Vacuoles found within the marginal cytoplasm of the syncytium and the cytotrophoblastic cells may be indicative of pinocytosis of fluid from the extracellular spaces.

The relationship of the syncytium to the basement membrane and foetal mesenchyme at the 54th day is shown in Pl. 6, fig. 6. The disappearance of the cytotrophoblast in many areas is interpreted as a senescent change, presumably associated with the exhaustion of this germinal layer in the continued production of syncytium. Where the cytotrophoblastic layer is deficient the syncytium again presents the characteristic pattern of microvilli, many of which are implanted on to the basement membrane by slightly expanded processes (Pl. 7, fig. 8). The basement membrane of the trophoblast is thickened and fibrillar in the later stages of pregnancies, and the foetal mesenchyme contains fewer cellular elements and a considerable amount of collagen. The confluence of the lacunae into the characteristic lattice-like pattern so well revealed in periodic acid-Schiff preparations is illustrated in Pl. 6, fig. 7: the lacunar material appears inspissated and embedded in the microvilli lining the walls of the cavity.

Maternal placental vessels

Beginning about the 15th day the subplacental trophoblast invades the decidual tissues along the path of the capillaries, some of which are later modified as the major placental vessels. The endothelium is eroded and replaced by trophoblastic epithelium. The term 'endotrophoblast' has been used to describe this lining epithelium since its syncytial or cytotrophoblastic character could not be determined with the light microscope. The nature of the endotrophoblast has been clarified by the use of the electron microscope. The cellular constitution of the limiting walls of the maternal blood channels within the placenta and the subplacenta and also of the major placental vessels during their course through the decidua has been traced at all levels and is illustrated by representative sections.

(1) *Within the chorio-allantoic placenta:*

The foetal and maternal blood come into closest apposition in the areas of so-called 'fine syncytium'. Here the maternal blood channels of the chorio-allantoic placenta are lined by endotrophoblast (Pl. 7, fig. 9), which appears to consist not of a true syncytium but of overlapping sheets of trophoblastic cells (Pl. 7, fig. 9). Cytotrophoblastic cells are identifiable in the chorio-allantoic placenta as late as the 25th day. They are always separated from the maternal blood stream by a layer of syncytial trophoblast. In the areas of 'coarse syncytium', which are not vascularized by foetal vessels, the fine structure of the lining of the maternal blood spaces resembles syncytial trophoblast (Pl. 7, fig. 10). However, the presence of ill-defined

membranes of considerable electron density within the endotrophoblastic wall suggests that the latter may also consist of overlapping sheets of cytoplasm and is, therefore, cytotrophoblastic rather than syncytial. The problem requires further study, however.

Small, extremely electron dense bodies are observed among the microvilli of the endotrophoblastic lining of the maternal blood spaces during the first half of pregnancy, especially in the transitional zone between the chorio-allantoic placenta and the subplacental syncytium (Pl. 7, figs. 9, 10). These bodies may have an angular profile, suggesting that they may have a crystalline structure. They are visible in the phase-contrast microscope as a delicate stippling of faintly osmiophilic material.

(2) *Within the subplacenta*

The large maternal placental vessels traversing the subplacenta near its edge continue to be lined by endotrophoblast which is syncytial in type (Pl. 8, fig. 11). Since these vessels are formed by the union of blood channels emerging from the base of the lobules of the chorio-allantoic placenta, it is likely that they are venous in character. The endotrophoblast appears vacuolated due to the presence of swollen mitochondria and dilated sacs and channels of the endoplasmic reticulum. Many of the latter contain a coagulum of amorphous material of moderate electron density. Residual cytotrophoblastic cells are found in relation to the basement membrane of these vessels until at least the 35th day of pregnancy. The endotrophoblast may rest on a basement membrane which separates it from the foetal (chorio-allantoic) mesenchyme (Pl. 8, fig. 11), or may merge insensibly with the subplacental syncytium with no intervening basement membrane (Pl. 8, fig. 12). The lacunae in immediate relationship to the maternal vessels are larger than in the rest of the subplacenta. They are separated by extremely attenuated septa of syncytial trophoblast and also contain amorphous material of varying electron density. This material, like that within the other subplacental lacunae, is strongly PAS positive. Large masses of PAS positive material within the outer portion of the walls of the large maternal veins piercing the subplacenta were described previously (Davies, *et al.* 1961): their relationship to the cellular components of the vascular wall could not be determined, however.

(3) *Within the necrotic zone of the basal decidua*

The maternal vessels within this zone of the basal decidua show striking modifications of their walls which consist of varying combinations of endotrophoblast with maternal endothelium. Four general types of vessels have been observed in which the walls consist of: (1) syncytial trophoblast alone, (2) maternal endothelium and syncytial trophoblast, (3) maternal endothelium and giant cytotrophoblastic cells, and (4) maternal endothelium alone.

The syncytial lining of the first type of vessel (Pl. 9, fig. 13) resembles in general features the endotrophoblast of the vessels at the level of the subplacenta. The vacuolation of the cytoplasm is very variable from one vessel to another. The microvilli at the luminal edge are branched and frequently appear swollen and hydropic. The cytoplasm contains ergastoplasm, swollen mitochondria and aggregates of

electron dense granules similar to those of the ergastoplasm in other sites. An irregularly thickened basement membrane intervenes between the endotrophoblast and the necrotic decidua. The latter is traversed by collagen fibres and contains amorphous masses of material which are also PAS positive.

The epithelium in immediate contact with the maternal blood in the second type of decidual vessel is the maternal endothelium (Pl. 9, fig. 14). The endothelial cells are imbricated and the plasma membranes show localized thickenings or terminal bars. The cytoplasm contains lipid inclusions. A thin basement membrane separates the endothelium from the endotrophoblast, and a second basement membrane separates the endotrophoblast from the necrotic decidual tissues. The syncytial cytoplasm resembles that of the large maternal vessels at the level of the sub-placenta (compare Pl. 8, fig. 11). The mitochondria are swollen and are distinguishable from the all-pervading sacs and channels of the endoplasmic reticulum by their thicker walls and by remnants of the cristae. In other vessels of the same type the endotrophoblast is less vacuolated (Pl. 10, fig. 15). The syncytium is implanted on the endothelial basement membrane by cytoplasmic processes or 'feet' (Pl. 9, fig. 14; Pl. 10, fig. 15), reminiscent of the podocytes of the visceral layer of Bowman's capsule in the renal glomerulus. The foot-processes enclose a labyrinthine subendothelial space which is continuous in many areas with the complex system of dilated sacs and channels within the endotrophoblastic cytoplasm. The transition from a vessel of the second type to one lined only by maternal endothelium (fourth type) is abrupt and at this point the two basement membranes become confluent (Pl. 10, fig. 16). The maternal vessels deeper within the basal decidua are generally composed solely of maternal endothelium resting on a thin basement membrane into which are inserted collagen fibres (Pl. 10, fig. 17).

In vessels of the third type (Pl. 11, fig. 19) the maternal endothelium is thickened. The endothelial cytoplasm is finely granular and contains mitochondria which may be small and electron dense with well-marked cristae or may be greatly swollen. The proximity of cells containing both types of mitochondria suggests that the swollen character of the organelles in some cells is not due to faulty fixation. Endothelial cells with swollen mitochondria also tend to have large cytoplasmic vacuoles as well as smaller vacuoles within the marginal cytoplasm adjacent to the maternal blood. The basal plasma membranes of the endothelial cells are highly irregular and rest on a very attenuated and apparently discontinuous basement membrane. External to the endothelium is a thick endotrophoblastic layer composed of large mononuclear cytotrophoblastic cells. They have been interpreted as trophoblastic since their fine structure clearly distinguishes them from decidual cells (see Pl. 12, fig. 21). It has not been possible to determine if the giant cytotrophoblastic cells form a single layer having limited areas of contact with the endothelial basement or if they are stratified. They are implanted on to the endothelial basement membrane by delicate foot processes, the terminal portions of which are expanded and rest in depressions hollowed out in the plasma membranes of the endothelial cells, the basement membrane being very attenuated or indistinguishable at the areas of contact. The spaces between the cytotrophoblastic cells are dilated and incompletely lined by microvilli. These spaces are, in turn, continuous with the complex subendothelial space enclosed by the branching processes of the cytotrophoblastic cells. Wisps of

coagulated material of moderate electron density are found within the intercellular spaces and the subendothelial space. The giant cytotrophoblastic cells are of two types, dark and light cells. The cytoplasm of the dark cells is electron dense and contains closely packed membranous and granular elements of the endoplasmic reticulum. The light cells have a cytoplasm of lower electron density which contains more widely scattered ergastoplasmic membranes and granules and many large vacuoles containing amorphous material. Smaller vacuoles are found within the marginal cytoplasm bordering the dilated intracellular spaces. A re-examination of the vessels of the basal decidua in histological preparations stained with haematoxylin and eosin showed that vessels of the third type may easily be recognized. The large cytotrophoblastic cells are visible as rounded vesicular cells in the outer part of the wall.

(4) *Within the residual zone of the basal decidua*

A residual layer of modified decidual cells separates the necrotic zone from the myometrium and persists until the end of pregnancy. Maternal placental vessels passing through this zone are for the most part lined only by maternal endothelium (fourth type; Pl. 10, fig. 18) or, more rarely, by endotrophoblast of the syncytial type (first type; Pl. 12, fig. 20). The endothelium or syncytium rests on a basement membrane by which it is separated from the decidual tissues. There is a wide subendothelial space containing wisps of collagen and masses of amorphous material. The latter is continuous with material of similar electron density within the complex infoldings of the surface plasma membranes of the decidual cells (Pl. 10, fig. 18). The plasma membranes of adjacent decidual cells are sinuous and interlock in a complex manner. The cells are separated by a substance of marked electron density which also extends into the bays resulting from the inflexion of the plasma membranes into the marginal cytoplasm (Pl. 12, fig. 21). The bays in some instances may be traced into continuity with large intracellular vacuoles lined by microvilli and containing amorphous material of lower electron density than that between the cells. These intra-cytoplasmic vacuoles may be very large, dwarfing the nucleus and may correspond with the large acidophilic and PAS positive masses observed by light microscopy in the giant decidual cells of the junctional region.

Present studies have not permitted a correlation between the varied cellular composition of the walls of the decidual vessels with their arterial or venous character. The differences in level at which the maternal endothelium reappears as the vessels are traced toward the myometrium probably reflect the extent of invasion of the decidua by the subplacental trophoblast. Moreover, the replacement of the endothelium of these vessels by trophoblastic elements certainly involves both arterial and venous channels. Careful injections of the vessels will have to be carried out before the problem can be solved satisfactorily.

Endoderm of the parietal wall of yolk sac and chorionic giant cells

The endodermal cells comprising the parietal wall of the inverted yolk sac and the underlying layer of chorionic giant cells are well developed and show an intimate morphological association throughout most of gestation. The cells of the parietal endoderm form a pseudostratified columnar epithelium having irregularly dilated intercellular spaces which confer upon this layer a characteristic tufted appearance

in the light microscope. The plasma membranes of the cells which face the decidual cavity are thrown up into long slender microvilli (Pl. 13, fig. 22). The decidual cavity contains a flocculum of precipitated material. The plasma membranes bordering on the dilated intercellular spaces are inflected into the cytoplasm to form a marginal system of recesses and bays containing amorphous material similar to that which fills the intercellular spaces. The plasma membranes of adjoining cells are thickened, forming attachment plates or terminal bars. The cytoplasm of the endodermal cells contains granular and membranous elements of the endoplasmic reticulum though these cells are not basophilic when studied in the light microscope. The mitochondria are small and scattered and the cytoplasm contains no lipid or other inclusions.

The chorionic giant cells are mononuclear cytotrophoblastic cells showing distinct limiting plasma membranes which interlock in a complicated manner (Pl. 13, fig. 22). The cytoplasm contains a few scattered mitochondria, islands of ergastoplasm which are predominantly perinuclear in position, large vacuoles and many homogenous inclusions of varying electron density. The vacuoles occur mainly near the plasma membrane, and in many cases are clearly derived by inflexion of the membrane. They contain flocculent material and many droplets, some of which are extremely electron dense. The homogenous inclusions are bounded externally by definite membranes but show no recognizable internal structure to link them with the mitochondria.

Reichert's membrane occupies the broad interval between the endodermal cells and the chorionic giant cells (Pl. 13, fig. 22). It is faintly fibrillar with no identifiable collagen. The substance of Reichert's membrane is prolonged into the dilated intercellular spaces of the endodermal layer, these extensions of PAS positive material being clearly visible in the light microscope (Davies *et al.* 1961). It is also continuous with the amorphous material contained within the superficial vacuoles of the cytoplasm of the chorionic giant cells.

DISCUSSION

The salient histological features of the subplacenta of the guinea-pig have been confirmed by the use of the electron microscope. The syncytial vacuoles or lacunae, the true nature of which could not be resolved in the light microscope, have been shown to be well differentiated spaces lined by microvilli. The lacunae arise as apparently isolated spaces within the subplacental syncytium in the early stages of pregnancy but communicate extensively in the later stages. They must probably be regarded as intracytoplasmic vacuoles rather than as modified extracellular spaces. Their true nature and that of the marginal extracellular space between the subplacental syncytium and the cytotrophoblast is, however, bound up with the difficult problem of the origin of the syncytium from the parent cytotrophoblastic layer. That protoplasmic sheets, essentially cytotrophoblastic in nature and separated by true extracellular spaces, can occur in the placenta is shown by the observations of Wislocki & Dempsey (1955*b*) on the lining epithelium of the maternal blood spaces in the chorio-allantoic placenta of the rat which consists of imbricated mononuclear or occasional binuclear cells. Preliminary observations reported in this paper suggest that the same may be true of the chorio-allantoic placenta of the guinea pig. In the light of these observations, the presence of the lacunae within the subplacenta

may reflect some peculiarity in the origin of the syncytium which deserves more careful study with the electron microscope.

The material within the lacunae corresponds with the PAS positive masses previously identified within the subplacental syncytium. The origin of this material is entirely speculative. Possible precursors of the material may exist in the form of the electron dense droplets or within the dilated ergastoplasmic sacs of the syncytial cytoplasm. The subplacental syncytium is also characterized by the paucity of mitochondria and the presence of large amounts of glycogen. The latter observation is a further example of the generalization of Wislocki, Deane & Dempsey (1946) that glycogen is abundant in parts of the placenta which are far removed from a source of blood supply and may therefore be characterized by a high rate of anaerobic glycolysis. Lipid is absent from the subplacental syncytium, suggesting that it is not concerned in the elaboration or storage of the steroid hormones. The cytotrophoblastic cells which give rise to the syncytium are characterized by their large nuclei, multiple skein-like nucleoli and their granular cytoplasm. These features may possibly be correlated with an active synthesis of ribonucleoprotein by these cells which are strongly basophilic after staining with the basic aniline dyes.

The fine structure of the syncytial trophoblast (endotrophoblast) which is in contact with the maternal blood entering either within the chorio-allantoic placenta or within the walls of the maternal placental vessels differs strikingly from that of the subplacental syncytium. The microvilli at the luminal surface, the swollen mitochondria, the dilated endoplasmic reticulum and the absence of glycogen are features of the endotrophoblast which mark it as an actively absorbing epithelium. It resembles the syncytial trophoblast on the surface of the human chorionic villi, as described in the electron microscope by Boyd & Hughes (1954) and by Wislocki & Dempsey (1955*a*).

Extensions of the subplacental trophoblast into the walls of the decidual vessels have been described in this paper and present a variety of interesting relationships between the endotrophoblast and the maternal blood. In the vessels immediately beneath the subplacenta, which are lined only by syncytial trophoblast, the relationship may be termed haemochorial as in the chorio-allantoic placenta. Deeper within the decidua the maternal endothelium is interposed between the endotrophoblast and the maternal blood stream, thus representing a haemoendothelial condition. In some cases the endotrophoblast is syncytial and in others is composed of giant cytotrophoblastic cells. In both cases the endotrophoblast is implanted on the endothelial basement membrane by cytoplasmic feet and, especially in the former case, strikingly resembles the conditions in the chorio-allantoic labyrinth of the cat (Dempsey & Wislocki, 1956). Complex modifications of the surface plasma membranes and swelling of the motochondria of the endotrophoblast have been described in this paper and may be tentatively correlated with the occurrence of fluid exchanges between the maternal and foetal organisms, with or without the intervention of the maternal endothelium. The walls of the decidual vessels remain very simple in relation to the residual zone of the basal decidua and consist of maternal endothelium alone or, more rarely, of syncytial trophoblast. The relationship of these vessels to the decidual cells, with a minimum of intervening mural elements, suggests that they may mediate important exchanges of materials between the maternal blood

and the decidual tissues. The electron dense material which encapsulates individual decidual cells, and which is strongly PAS positive in the light microscope, also extends into the marginal bays and intracellular vacuoles of the cells, suggesting that it may be elaborated by them. The encapsulating material also extends into the subendothelial space around the large decidual vessels. The replacement of the endothelium of the large maternal placental vessels within the decidua by large cells of unknown though presumably trophoblastic origin has been described in many rodents (Duval, 1890; Mossman, 1937). These vessels have not, however, so far been studied with the electron microscope.

The fine structure of the endodermal cells of the parietal walls of the inverted yolk sac and of the underlying layer of chorionic giant cells are consistent with the view that these layers are involved in the absorption of materials from the decidual cavity. The passage of such materials may be modified by the presence of Reichert's membrane between the two layers. Evidence that this membrane may arise as product of the chorionic giant cells has been presented. The membrane in the guinea-pig differs from that of the rat is being less compact and less fibrillar (Wislocki & Dempsey, 1955*b*). It also differs from visceral walls of the yolk sac which is fenestrated, more coarsely fibrillar and receives the terminations of collagen and reticular fibres reaching it from the exocoelomic mesenchyme (Dempsey, 1953).

The details of fine structure displayed by the cellular and syncytial elements of the placenta, both in the region of the subplacenta and in the walls of the decidual vessels, clearly illustrate the limits of the Grosser classification when the sites of transfer of materials of physiological importance between the maternal and foetal organisms are being considered. The electron microscope so far has scarcely been exploited in the elucidation in these complexities of placental structure.

SUMMARY

1. The fine structure of the guinea-pig subplacenta and the related parts of the chorio-allantoic placenta and basal decidua is described in the early and middle stages of gestation.

2. The cytotrophoblastic layer of the subplacenta is characterized by the dilated intercellular spaces, the finely granular cytoplasm, the numerous mitochondria, the large size of the nuclei relative to the cytoplasm, the multiple skein-like nucleoli, and the absence of glycogen and lipid. The subplacental syncytium is characterized by the relative paucity of mitochondria, the scattered areas of ergastoplasm, many electron dense droplets, large accumulations of glycogen and an absence of lipid. In addition, the syncytium is traversed by a complex and possibly anastomosing system of lacunae lined by microvilli and containing electron dense material which is strongly periodic acid-Schiff positive.

3. The highly modified walls of the maternal placental vessels are described at different levels. The lining of the maternal blood channels within the foetal placenta consists of overlapping sheets of chorionic epithelium. The lining of the vessels (endotrophoblast) as they pass through the subplacenta is syncytial. The walls of the vessels within the necrotic zone of the basal decidua consists of either (1) syncytial trophoblast alone, (2) maternal endothelium and syncytial trophoblast, (3) maternal endothelium and giant cytotrophoblastic cells, or (4) maternal endothelium alone.

4. The cells of the residual zone of the basal decidua are individually surrounded by a capsule of electron dense material which extends into the marginal bays and intracellular vacuoles of the cytoplasm. The relationship of these cells to the maternal vessels is described.

5. The endodermal cells of the parietal wall of the inverted yolk sac and the underlying Reichert's membrane and layer of chorionic giant cells are described.

6. The possible functional significance of the morphological specializations described in the subplacenta and in the vascular walls is discussed.

Grateful acknowledgement is made to Miss Sonia Michelson for technical assistance.

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EXPLANATION OF PLATES

The following abbreviations are used in the figures: B, bay of decidual cell; BM, basement membrane; CY, cytotrophoblast; DR, droplets; D, decidua; DC, decidual cell; DS, decidual space or cavity; DT, dark trophoblastic cell; DV, decidual vacuole; E, endoplasmic reticulum; EN, endothelium; ET, endotrophoblast; FM, foetal mesenchyme; FP, foot-process; FV, foetal vessel; G, glycogen; H, cell inclusion in chorionic giant cell; IC, intercellular space; L, syncytial lacuna; LT, light trophoblastic cell; M, mitochondrion; MV, maternal vessel; N, nucleus; PE, endoderm of parietal yolk sac; PM, plasma membrane; RM, Reichert's membrane; S, subplacental syncytium; ST, syncytial septum; SY, chorio-allantoic syncytium; TV, vacuole of chorionic giant cell; T, terminal bar. With the exception of Pl. 1, fig. 1, all figures are electron micrographs. All tissues were fixed in Dalton's fluid unless otherwise stated.

PLATE 1

Fig. 1. Subplacenta of the guinea-pig at the 25th day of gestation, drawn from a section viewed in the phase-contrast microscope. The cytotrophoblastic layer is multilaminar and rests on a thin basement membrane which separates it from the foetal mesenchyme. The intercellular spaces of this layer are dilated. The subplacental syncytium appears darker than the cytotrophoblast and contains vacuoles or lacunae and many small osmiophilic droplets. (Drawing by Mrs B. M. Velick.) $\times 500$.

Plate 2

Fig. 2. Subplacenta at about the 25th day from a typical area enclosed by black lines in Pl. 1, fig. 1. The cytotrophoblastic cells are characterized by their large nuclei, multiple skein-like nucleoli, dilated intercellular spaces, scattered mitochondria and granular cytoplasm. Terminal bars are found between adjacent cytotrophoblastic cells and between the plasma membranes of these cells and that of the syncytial trophoblast. Several large lacunae lined by microvilli are shown within the syncytium. ×5000.

Plate 3

Fig. 3. Subplacenta at about the 35th day. The cytotrophoblastic layer is reduced to one cell in thickness and has disappeared in one area (Y), where the syncytium rests on the basement membrane by delicate cytoplasm or foot-processes. Similar processes of the syncytium also project into the marginal extracellular space (X) between this layer and the cytotrophoblastic layer. The true syncytial nature of the subplacental syncytium is evident. ×3000.

Plate 4

Fig. 4. Details of the subplacenta syncytium at about the 37th day. The cytoplasm contains a few scattered mitochondria, islands of ergastoplasm, numerous electron dense droplets and large accumulations of glycogen. The larger lacunae are lined by microvilli and contain material of medium electron density. Other lacunae have smooth walls and are filled with more electron dense material. ×5000.

Plate 5

Fig. 5. Details of the junction between the cytotrophoblast of the subplacenta (to the right) and the syncytium (to the left). 37th day. The marginal extracellular space between the two layers (X) is narrow, contains small processes of other cells and is continuous with the dilated intercellular spaces between the cytotrophoblastic cells. These spaces are partially lined by microvilli derived from the limiting plasma membrane of the syncytium. The cytoplasm of the cytotrophoblastic cells is granular with scattered vesicles. The mitochondria of these cells are larger than those of the syncytium. The syncytial cytoplasm contains scattered mitochondria, masses of glycogen and droplets of varied electron density. ×11,000.

Plate 6

Fig. 6. Subplacenta at about the 54th day. A group of cytotrophoblastic cells occupy the upper part of the figure. Elsewhere the syncytium rests directly on the basement membrane by small foot-processes. Terminal bars are found where the plasma membranes of the cytotrophoblastic cells and of the syncytium come into contact. The basement membrane of the trophoblast is cut tangentially and is fibrillar. ×4000.

Fig. 7. Details of the subplacenta syncytial lacunae (54 days). The lacunae are filled with dense material in which are embedded the lining microvilli. The lacunae appear to communicate to some extent and may form a confluent system. Palade's fluid. ×6000.

Plate 7

Fig. 8. The basement membrane of the trophoblast is cut tangentially and appears amorphous. In it are embedded the foot-processes of the subplacental syncytium which enclose a labyrinthine extracellular space. Palade's fluid. ×10,000.

The following figures (9 to 20) illustrate the character of the epithelium lining the maternal blood channels, beginning within the chorio-allantoic placenta and proceeding toward the myometrium.

Fig. 9. Area of 'fine syncytium' from the chorio-allantoic placenta at about the 35th day. The lining of the maternal blood channels is made up of the imbricated trophoblastic cells and is not syncytial. Irregularly dilated spaces within the cytoplasm (X) indicate intercellular spaces. Small electron dense bodies are embedded between the microvilli at the luminal edge of the trophoblast. ×2500.

Fig. 10. Area of 'coarse syncytium' (no foetal vessels) within the chorio-allantoic placenta at the 35th day. The lining of the maternal blood spaces may consist of syncytial trophoblast or of imbricated sheets of cytotrophoblastic cells. The linear dense areas within the cytoplasm (at X) may represent the plasma membranes of overlapping cells. The electron dense bodies between the microvilli are angular in profile, suggesting a crystalline structure. ×10,000.

PLATE 8

Fig. 11. Wall of a large maternal placental vessel passing through the subplacenta (about 35 days). The vessel is lined by a thick layer of syncytial trophoblast (endotrophoblast). The syncytial cytoplasm appears vacuolated due to the presence of swollen mitochondria and dilated vesicles and tubules of the endoplasmic reticulum. Many of the dilated spaces of the endoplasmic reticulum contain amorphous material (at X). A group of cytotrophoblastic cells is shown in relation to the basement membrane. ×4000.

Fig. 12. Dilated syncytial lacunae within the subplacenta in immediate relation to a large maternal vessel (about 35 days). The syncytial trophoblast separating the lacunae are extremely attenuated. The lacunae contain a flocculum of precipitated material and masses of dense material (X). ×4000.

PLATE 9

Fig. 13. Wall of a large maternal vessel (first type) within the necrotic zone of the basal decidua close to the subplacenta (45 days). The endotrophoblastic lining of the vessel is syncytial. The cytoplasm contains swollen mitochondria, occasional tubular and vesicular elements of the endoplasmic reticulum, and dense accumulations of granules. ×10,000.

Fig. 14. Portion of wall of large maternal vessel (second type) within the necrotic zone of the basal decidua (45 days). The vessel is lined by maternal endothelium external to which is syncytial trophoblast (endotrophoblast). A thin basement membrane lies between the endothelium and the endotrophoblast. The marginal cytoplasm of the latter is inserted into the endothelial basement membrane by foot-processes enclosing an extracellular space. This space communicates at some points (X) with the dilated cisterns and tubules of the endoplasmic reticulum. ×10,000.

PLATE 10

Fig. 15. Portion of wall of a maternal vessel (second type) within the necrotic zone of the basal decidua (45 days). The endotrophoblastic cytoplasm is less vacuolated than in Pl. 9, fig. 14. The foot-processes of the endotrophoblast related to the endothelial basement membrane are shown. ×14,000.

Fig. 16. Wall of maternal placental vessel (second type) within the necrotic zone of the basal decidua (45 days). The wall consists of maternal endothelium and of endotrophoblast which is syncytial. The dilated tubules and cisterns of the endoplasmic reticulum are very conspicuous in the endotrophoblast. At the lower edge of the figure the endotrophoblast fades out, leaving only the maternal endothelium, and the two basement membranes (that of the endothelium, that of the endotrophoblast) become confluent. ×2500.

Fig. 17. Wall of maternal vessel (fourth type) within the basal decidua consisting solely of maternal endothelium. The plasma membranes of adjoining cells interlock with no visible terminal bars. Lipid inclusions are found within the endothelial cytoplasm. ×8000.

Fig. 18. Wall of maternal vessel within the residual zone (normal though modified) zone of the basal decidua, close to the myometrium. An extensive extracellular space intervenes between the basement membrane of the maternal endothelium and the plasma membranes of the decidual cells. The latter are inflected into the decidual cytoplasm in the form of bays and are filled with amorphous material. Wisps of collagen and similar accumulations of amorphous material occupy the sub-endothelial space. ×8000.

PLATE 11

Fig. 19. Wall of maternal placental vessel (third type) within the necrotic zone of the basal decidua (46 days). The maternal endothelium is thick and finely granular. One endothelial cell contains swollen mitochondria and large vacuoles. External to the endothelium is the endotrophoblast made up of several layers of giant cytotrophoblastic cells. These are of two types: light

and dark, based on the electron density of the cytoplasm and the development of the endoplasmic reticulum. Foot-processes of the endotrophoblastic cells are implanted on to the endothelial basement membrane (at F). The labyrinthine extracellular space between the processes is continuous with the dilated intercellular spaces between the giant cytotrophoblastic cells. Vacuoles are present within the marginal cytoplasm of the endothelium and the light cytotrophoblastic cells. ×10,000.

PLATE 12

Fig. 20. Wall of maternal vessel within the residual zone of the basal decidua (45 days). The lining endotrophoblast is syncytial and is very electron dense, perhaps the result of degenerative changes. It is related externally to normal decidual cells, a wide extracellular space intervening (see Pl. 10, fig. 18). ×2500.

Fig. 21. Decidual cells within the residual zone of the basal decidua (36 days). The plasma membranes are sinuous and interlock in a complex manner. They are inflected into the cytoplasm as recesses and bays which contain material of marked electron density. These bays in some cases may be traced into continuity with large intra-cytoplasmic vacuoles lined by microvilli and containing granular material of low electron density. The decidual cytoplasm contains a few mitochondria, scattered electron dense inclusions (pigment?) and glycogen. ×2500.

PLATE 13

Fig. 22. Section through the layer of endodermal cells comprising the parietal wall of the inverted yolk sac and through the underlying layer of chorionic giant cells. The surface of the endoderm facing the decidual cavity is thrown up into long microvilli. The intercellular spaces of this layer are dilated and contain amorphous material continuous with Reichert's membrane. The giant cytotrophoblastic cells contain perinuclear aggregations of endoplasmic reticulum, homogeneous bodies of varying electron density enclosed by definite membranes, scattered mitochondria, and large vacuoles containing small electron dense inclusions. ×10,000.

DAVIES, DEMPSEY AND AMOROSO—THE SUBPLACENTA OF THE GUINEA PIG

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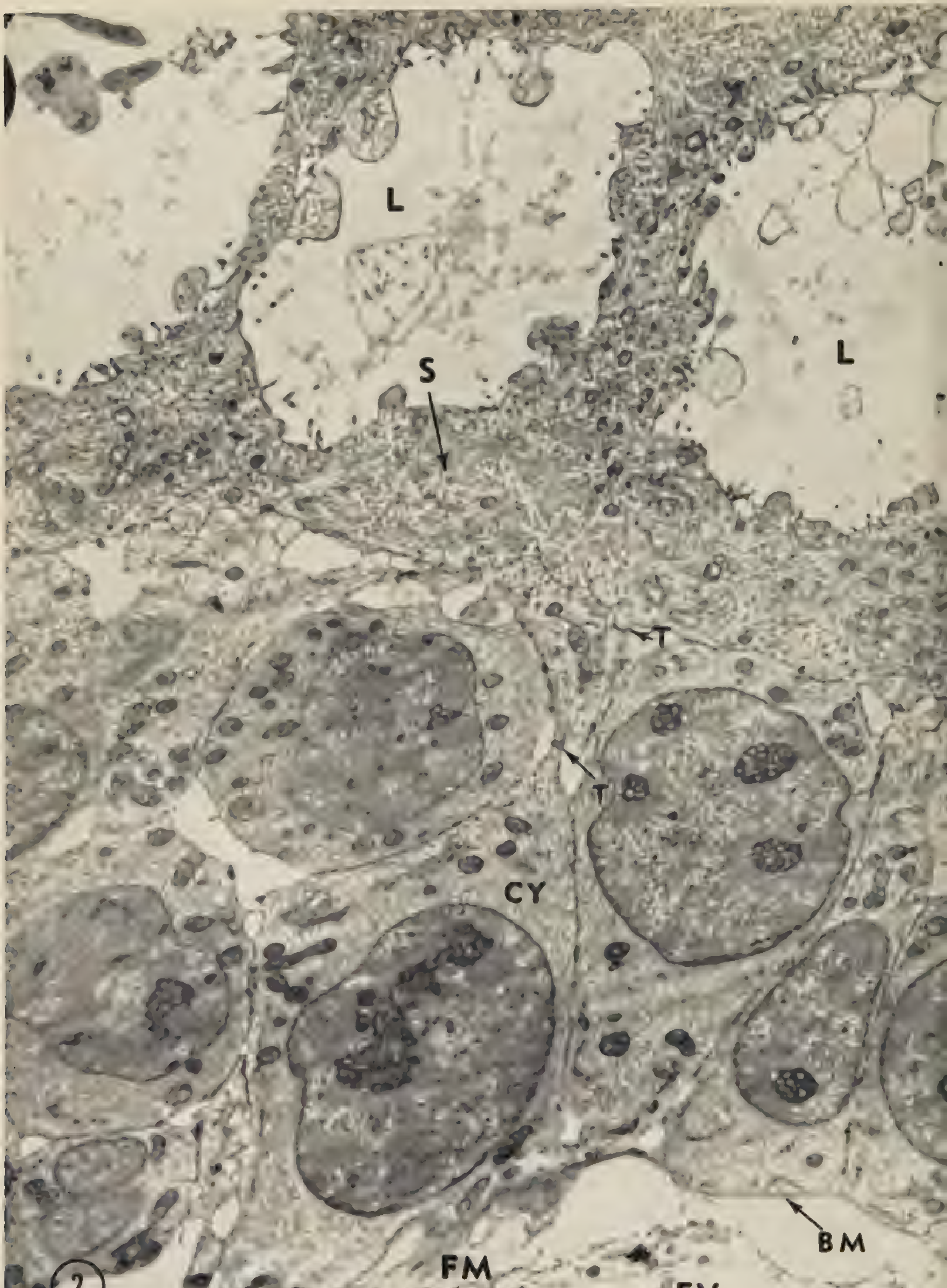

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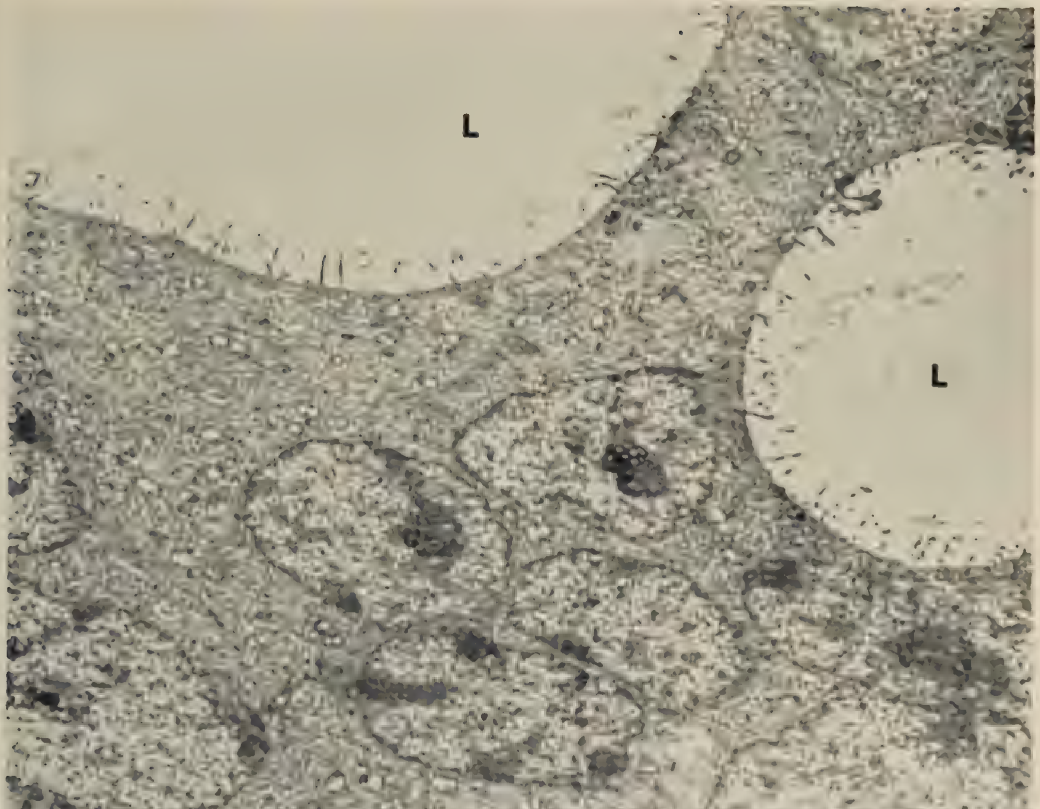

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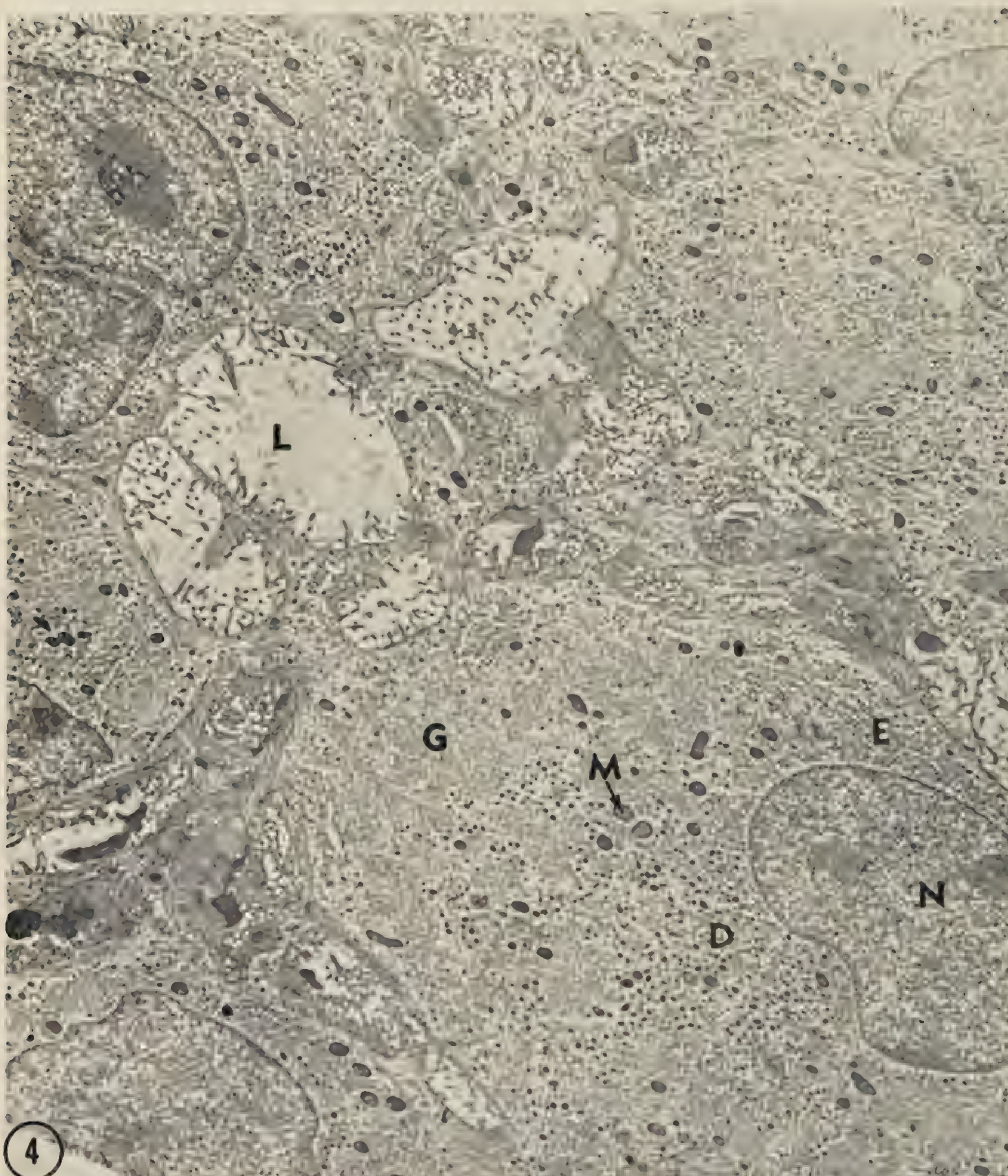

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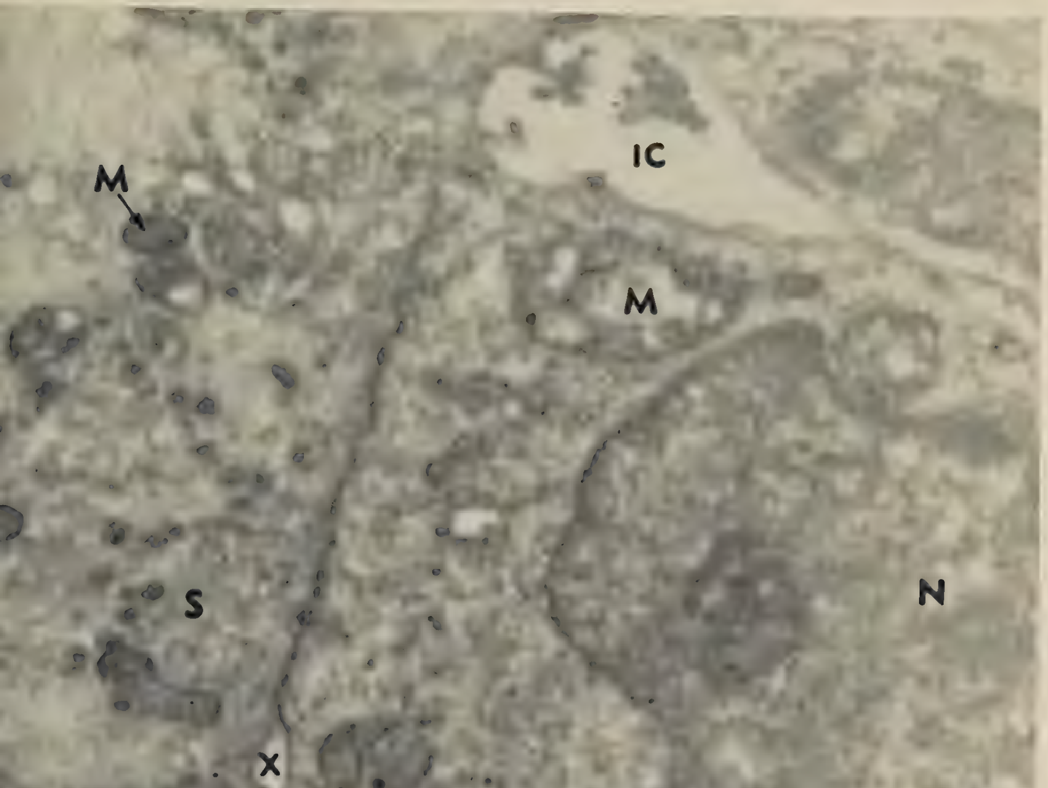

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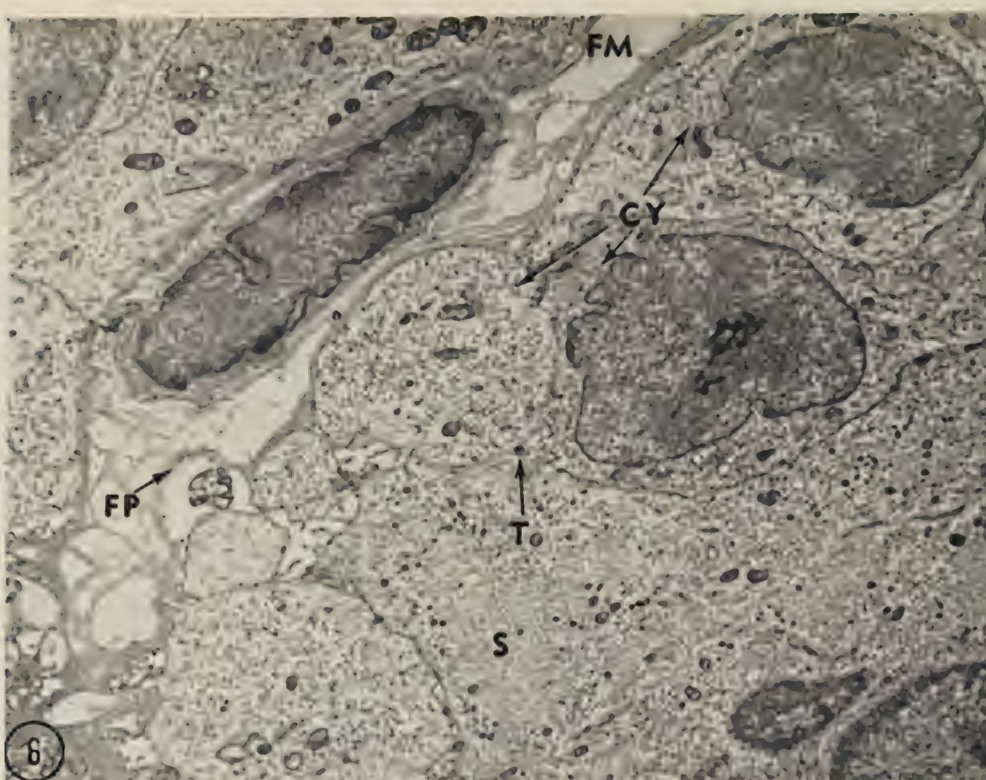

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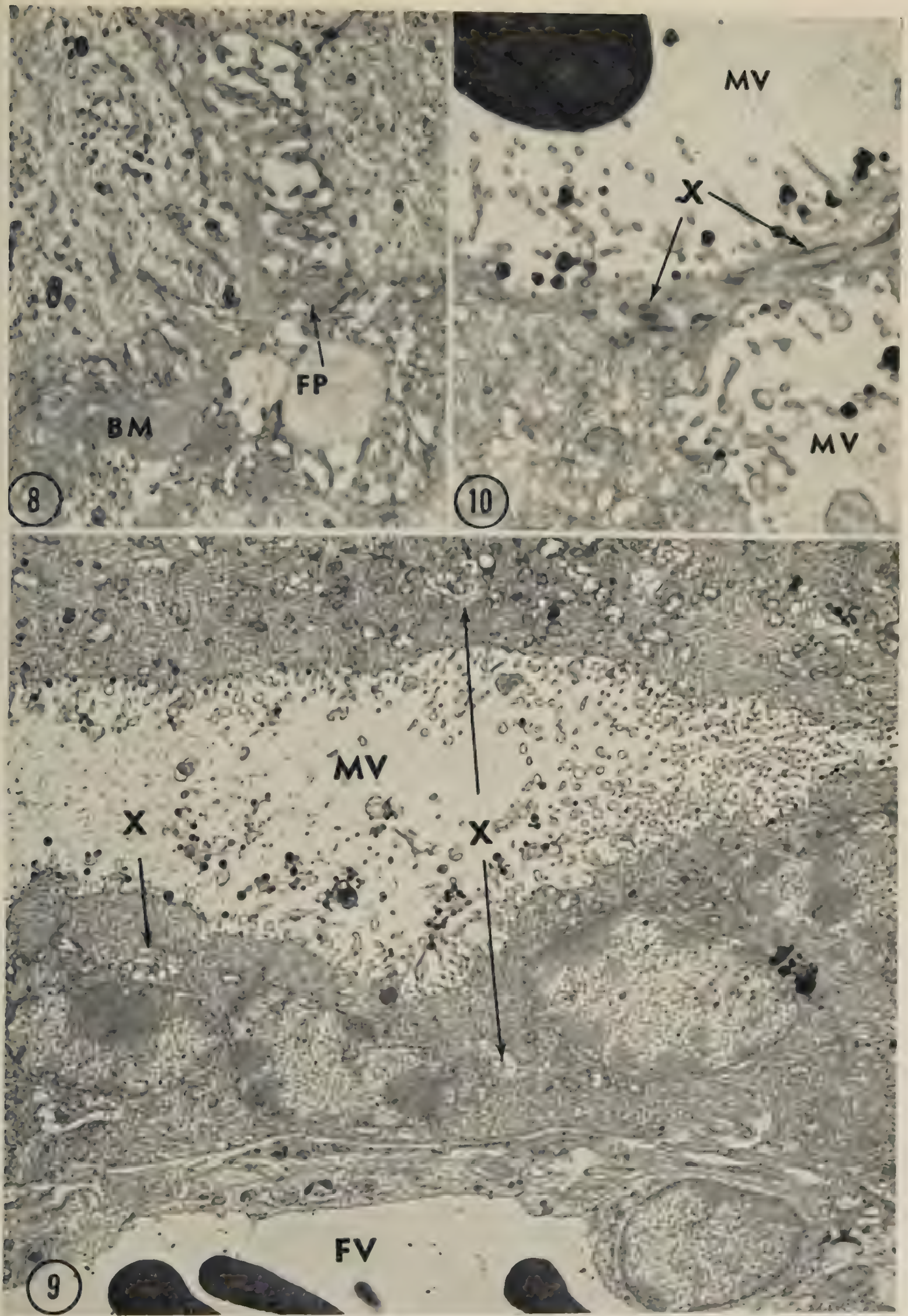

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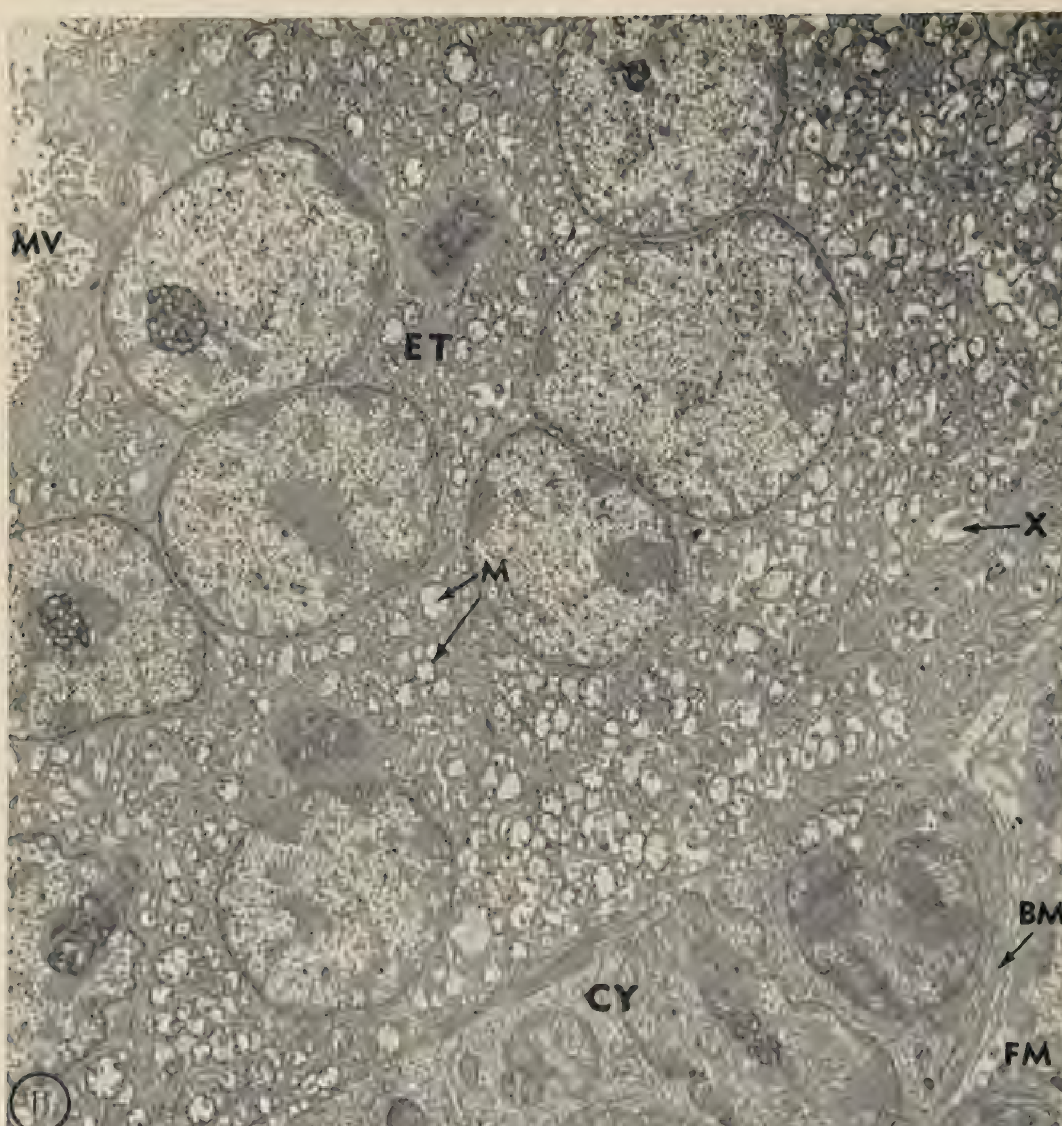

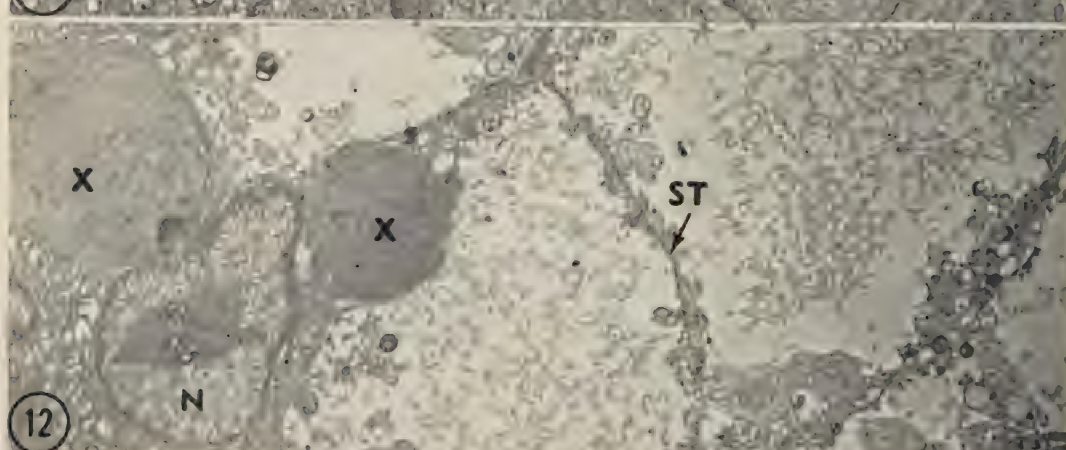

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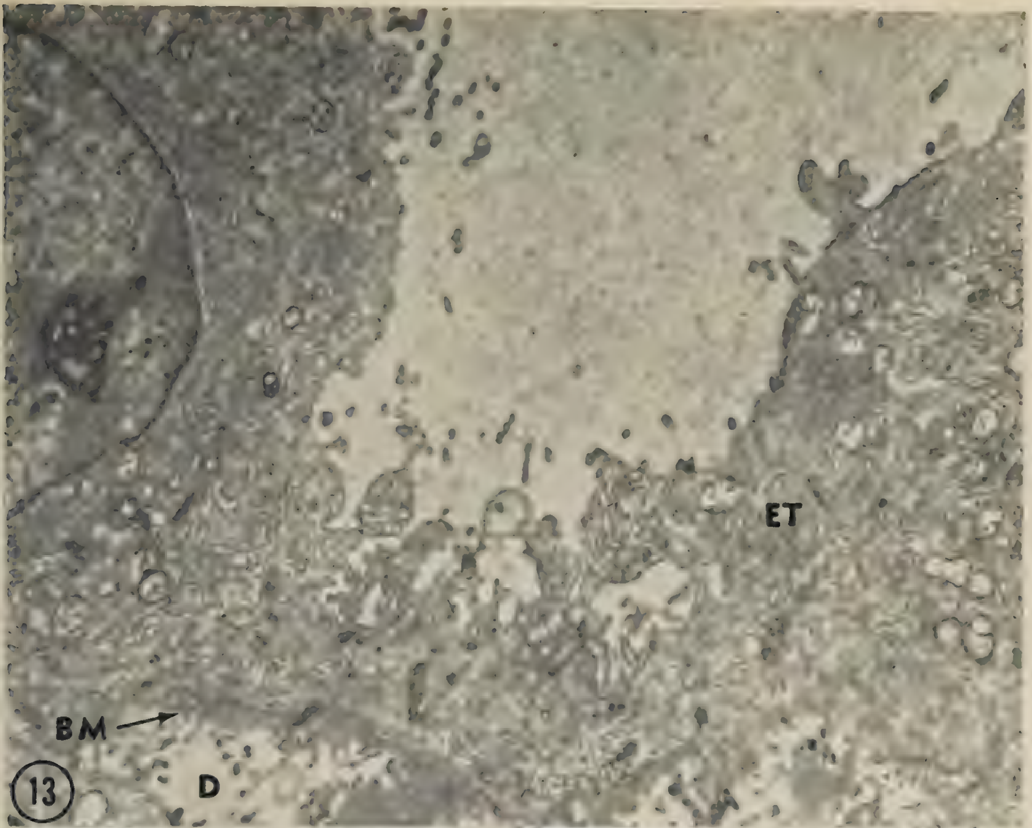

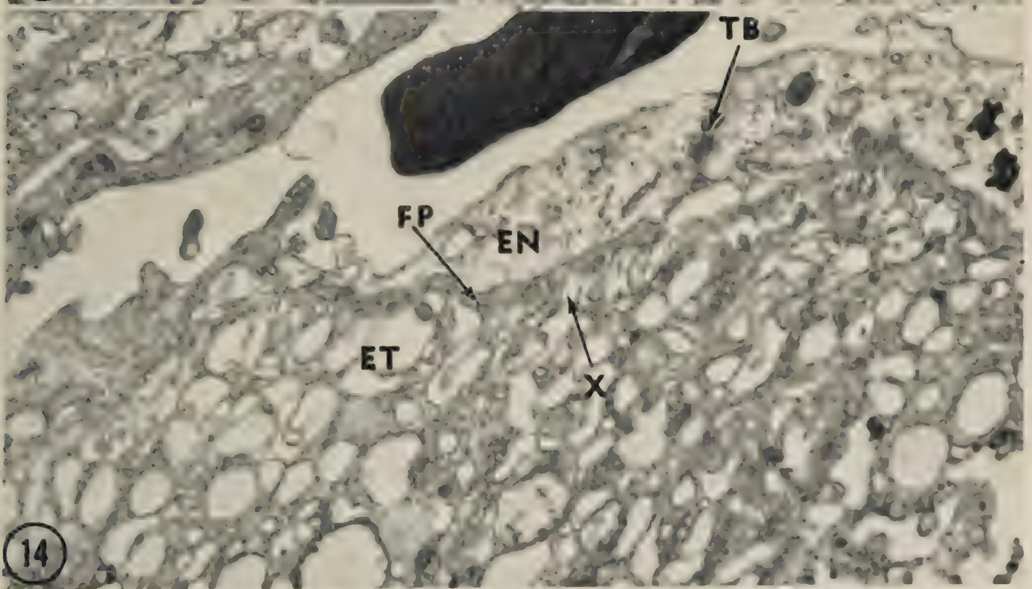

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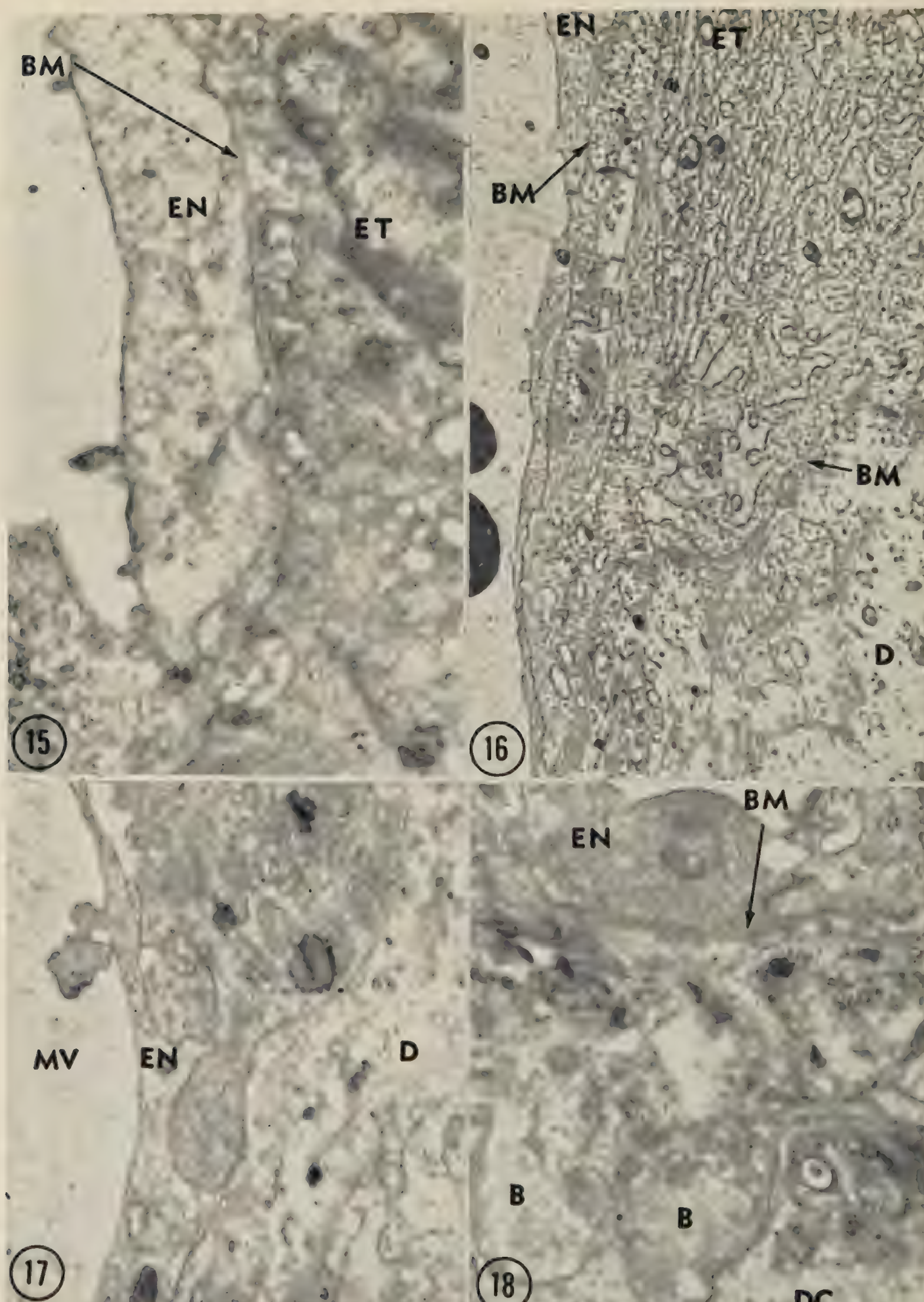

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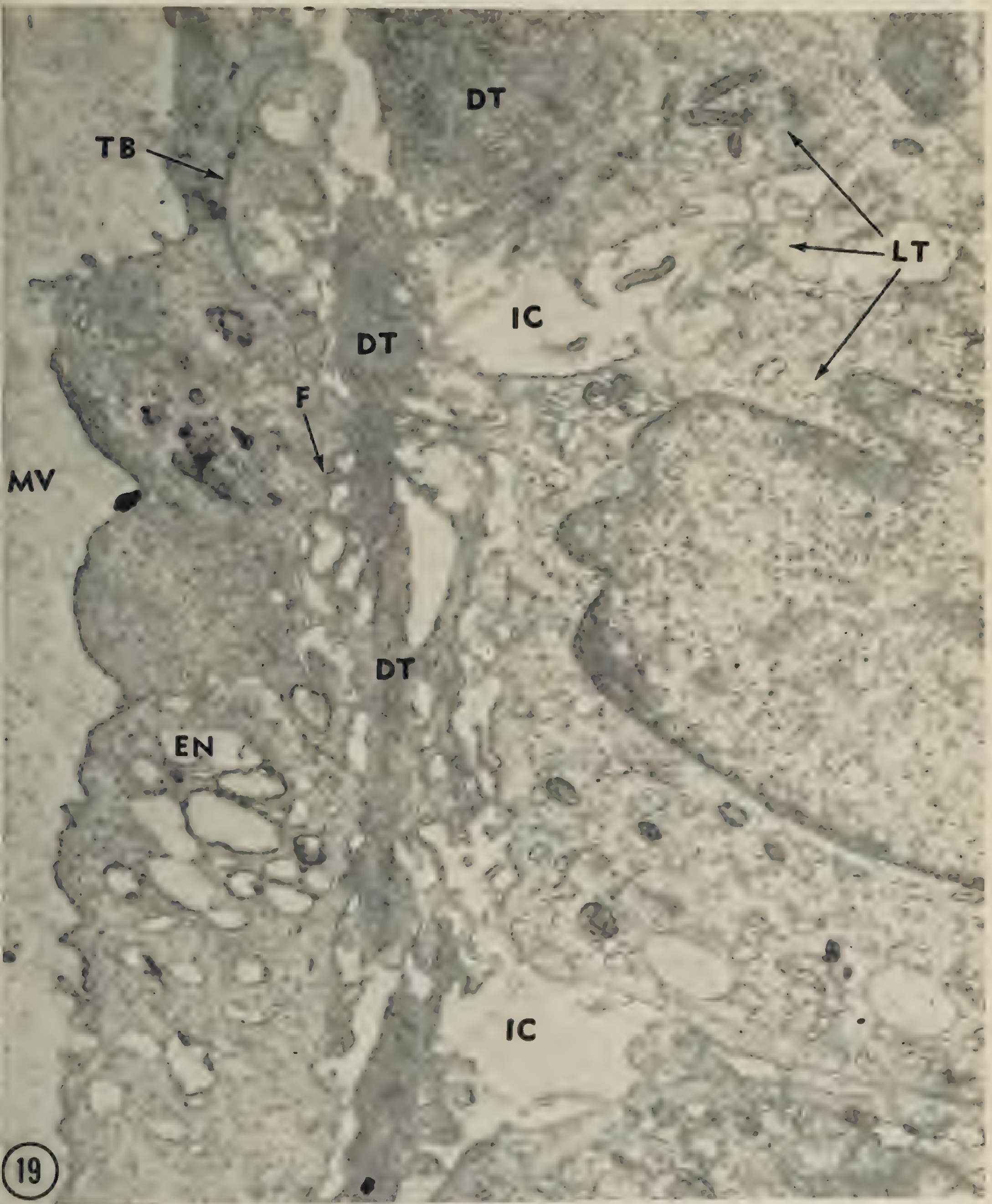

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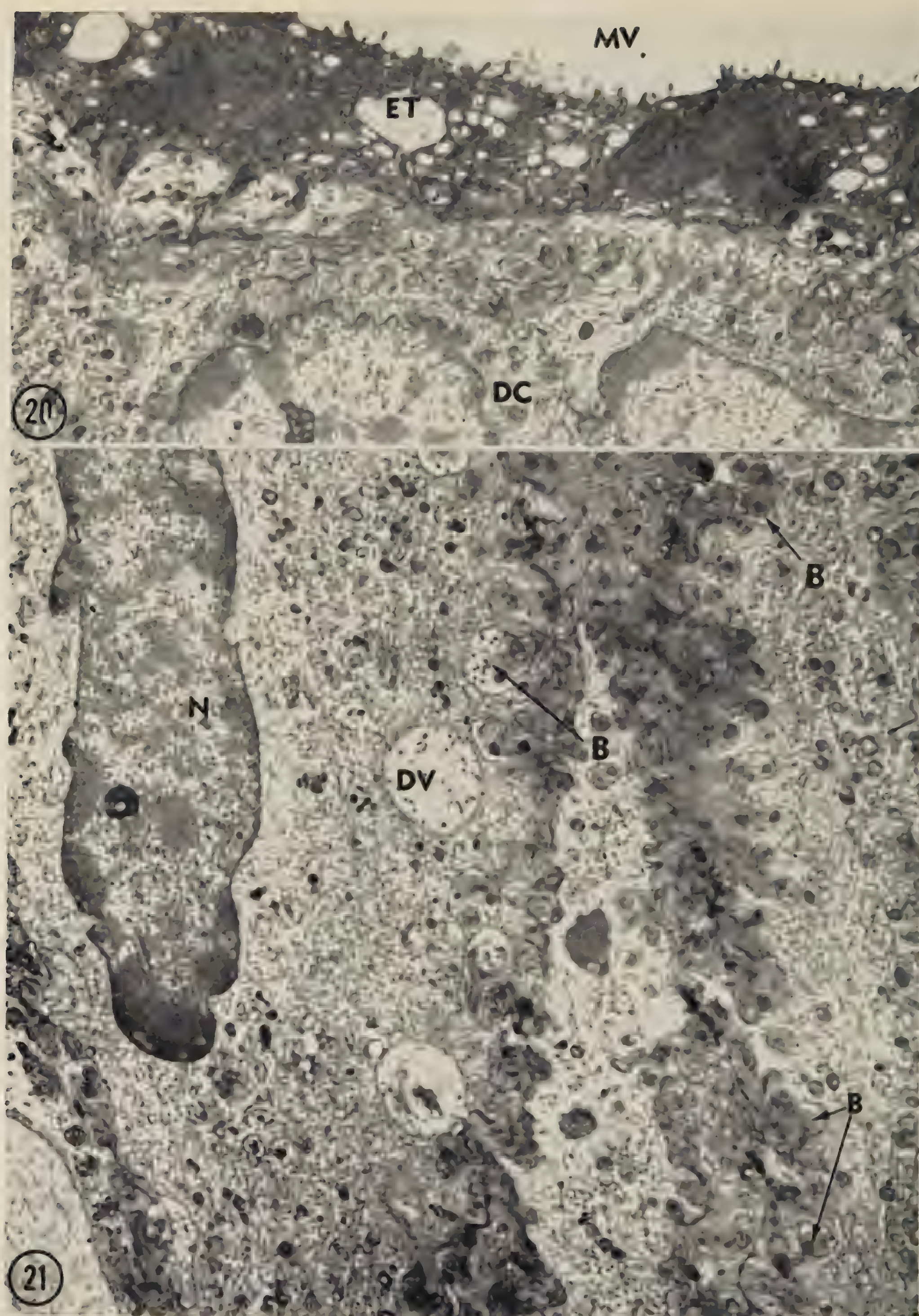

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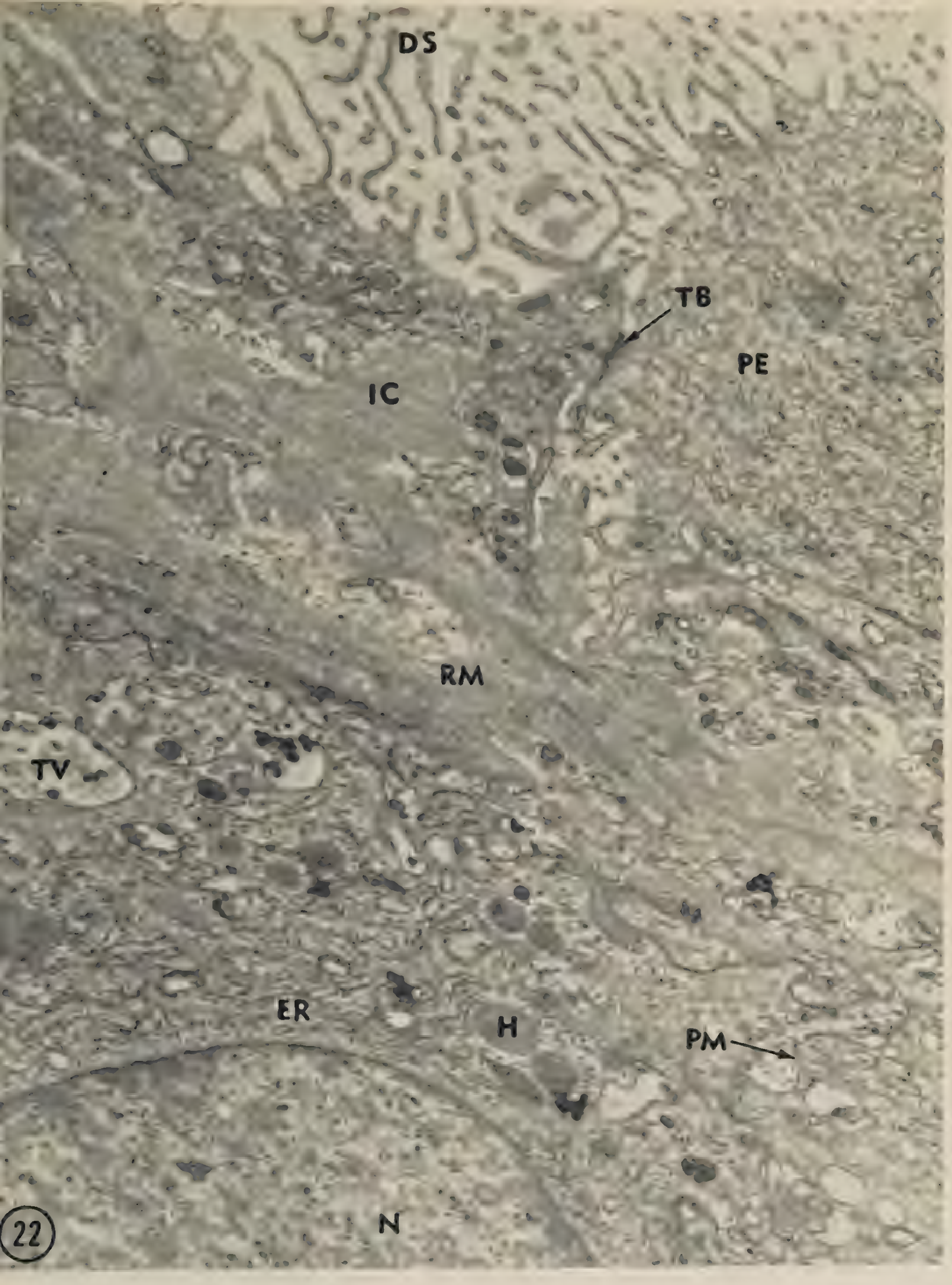

DAVIES, DEMPSEY AND AMOROSO—THE SUBPLACENTA OF THE GUINEA PIG

THE ADRENAL GLANDS OF THE FERRET, *MUSTELA PUTORIUS*

By R. L. HOLMES
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INTRODUCTION

It is apparent from the extensive literature dealing with the anatomy of mammalian adrenal glands (see reviews by Bourne, 1949; Bachmann, 1954) that there are considerable variations in both gross and microscopic structure among animals of different species. Even within a single species the histological characteristics of the glands are not constant. In cats, for example, Bennett (1940) found that the zone of lipid-poor cells forming the inner zona fasciculata (zone three, or post-secretory zone, in his terminology) was absent in young animals, and that the lipid-rich outer zona fasciculata was often wider in glands from female animals than in those from males. Lobban (1952) who also worked with cats, found that she could correlate changes in the width of the inner layers of the cortex with stages of sexual activity.

Few studies are available of the adrenal glands of members of the family *Mustelidae*. Meckel (1806) and Kolmer (1918) examined the adrenals of the stone martin, and the latter author also examined those of the weasel. A number of early workers (see Bourne, 1949) studied the adrenal glands of the otter. Bourne (1949) appears to be the only author who has published details of the histology of the glands of the ferret *Mustela putorius*, and based his short account on specimens from three mature male animals. The present paper deals with the anatomy, histology and certain histochemical characteristics of glands from a larger series of ferrets, mostly normal mature females.

MATERIALS AND METHODS

The adrenal glands from twenty-eight normal healthy mature female ferrets, killed at various times during the oestrous cycle have been examined, together with glands from four male animals. All were killed by an overdose of Nembutal given intraperitoneally. The adrenals were immediately removed and placed on filter paper moistened with normal saline, dissected free of fat and connective tissue and weighed on a torsion balance to the nearest milligram. They were then fixed in formol-saline at 3–4° C. for 18 hr. After fixation the upper and lower poles of the glands were cut away with a razor blade, and frozen sections 10 μ in thickness cut from the centre block. These sections were mounted on clean slides, and allowed to dry at room temperature. The histochemical techniques listed below were carried out using the methods given by Pearse (1960):

Lipids (Oil Red O).

Non-specific esterase (α-naphthyl acetate method, with 5 nitro-anisidine diazotate as substrate).

Alkaline phosphatase (Gomori method).

Acid phosphatase (Gomori method).

Cholinesterase (Modified Koelle method, Coupland & Holmes 1957); sections were incubated 3–36 hr. at pH 5·4.

The residual portions of the glands were washed, dehydrated and embedded in paraffin. Sections were cut at 6μ and stained by either haematoxylin and eosin or Masson's trichrome. Glands from three animals were fixed in formol-dichromate to demonstrate the chromaffin reaction.

The general anatomy and blood supply of the glands was examined in five animals in which the abdominal arteries had been injected with latex through the aorta, by a technique described previously (Holmes & Wolstencroft, 1959). After fixation these specimens were dissected under a binocular microscope.

The body weight and weight of the ovaries and adrenal glands of a number of normal healthy female ferrets which were not used for histological studies were also recorded, and these data are included in Table 1. A hundred and thirty-five animals were examined macroscopically to determine the incidence of accessory adrenal tissue.

TERMINOLOGY

The terms used in the description of the cortex are those of Nicander (1952), namely: Outer connective tissue capsule. Zona glomerulosa. Zona intermedia. Zona fasciculata: (*a*) outer zona fasciculata, (*b*) inner zona fasciculata. Zona reticularis. Zona juxtamedullaris. Medullary connective tissue capsule.

OBSERVATIONS

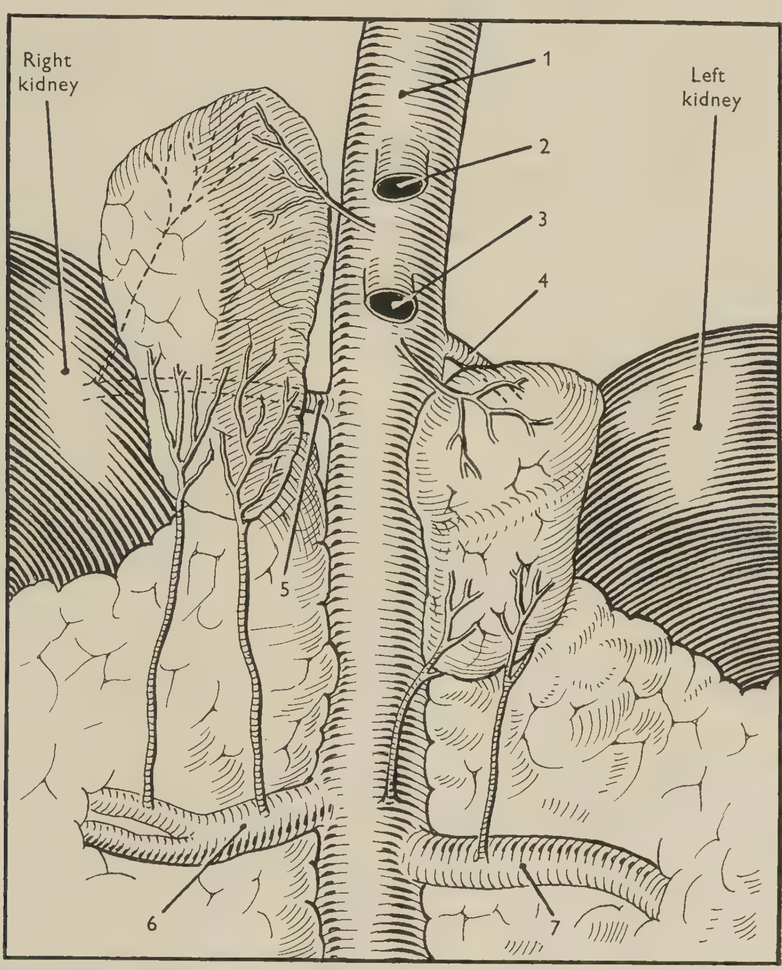
General anatomy of the glands

The adrenal glands of the ferret are embedded in fatty tissue adjacent to the upper medial borders of the left and right kidneys, but the exact position of the glands varies from one animal to another. The left gland usually lies close to the left side of the abdominal aorta, caudal to the origin of the superior mesenteric artery and rostral to the left renal artery, in relation to the upper third of the kidney. The gland is oval, 6–8 mm. in length and usually grooved across its ventral surface by the adreno-lumbar vein, which crosses it to enter the vena cava. In one specimen this vein crossed the dorsal surface of the gland.

The right gland usually lies more rostral than the left, close to the right side of the aorta, level with or rostral to the point of origin of the superior mesenteric artery. The gland is always related ventrally to the posterior vena cava which either overlaps the medial half of the gland, or overlies it completely. The right gland is usually more elongated than the left, measuring between 8 and 11 mm. in length. The upper pole is often larger than the lower and the ventral surface flattened or concave where it is in contact with the vena cava. The gland is occasionally grooved by the right adreno-lumbar vein. In one animal the right adrenal measured only 6·5 mm. in length, while the left gland measured 8 mm.; but in this case a large accessory adrenal, 2·8 mm. in length, was embedded in the fat around the hilum of the right kidney, and probably accounted for the reduced size of the main gland.

Blood supply

The pattern of the arterial blood supply to the glands varied in each of the five latex-injected animals. The left adrenal was found to be supplied by two, three or four main vessels, which divided into smaller branches as they approached the



Text-fig. 1. Drawing of a typical arrangement of the arterial blood supply to the adrenal glands. The posterior vena cava, which overlaid the medial shaded area of the right gland, has been removed. (1, aorta; 2, coeliac artery; 3, superior mesenteric artery; 4, left adreno-lumbar artery; 5, right adreno-lumbar artery; 6 and 7, right and left renal arteries.)

gland. One or two of the main arteries arose from the left renal artery; the remainder were direct branches from the aorta, at some level between the origin of the coeliac artery and a point caudal to the origin of the left renal artery. The left gland of one animal received a branch from the left adreno-lumbar artery.

The right gland was found to be supplied by three, four or five separate vessels.

One or two of these invariably arose from the right renal artery, and in three of the five animals the right adreno-lumbar artery provided branches; others came direct from the aorta at points lying between the origin of the coeliac and left renal arteries. A typical arrangement of adrenal arteries is shown in Text-fig. 1.

Weights of the glands

Table 1 gives data derived from the weights of the adrenal glands of normal female ferrets. The animals were divided into three groups, the first of which consisted of thirty-six young (first season) anoestrous females, all apparently healthy, which were killed in October. The second group consisted of eleven animals which were killed in January, February or March. As judged by vulval swelling, most of these animals were anoestrous, but some showed very slight vulval swelling possibly indicative of early pro-oestrus. In the absence of advanced vulval changes, it was decided to group these animals together, as all were likely to come into oestrus within a relatively short time compared with the ferrets of group I, which would be expected to pass through a minimum of 3–4 months in an anoestrous condition before the breeding season. Animals of the third group were in late pro-oestrus or full oestrus as judged by vulval swelling.

Table 1. *Mean adrenal, ovarian and body weight of normal female ferrets*

Left adrenal (mg.)	Right adrenal (mg.)	Paired adrenals (mg.)	Paired ovaries (mg.)	Body weight (g.)
Group I. (36 anoestrous animals killed in October)				
51·3	55·5	107·1 (S.E.M. 2·46)	67·7	653
Group II. (11 anoestrous or early pro-oestrous animals killed January-March)				
37·7	41·0	79·1 (S.E.M. 3·6)	111·1	741
Group III. (9 animals killed in late pro-oestrus or full oestrus)				
53·0	57·5	111·0 (S.E.M. 8·2)*	121·0	747

* The high S.E.M. for group III was largely due to one animal, whose paired adrenal weight was 168 mg. There was no apparent reason for excluding this animal from the series.

No significant difference was found in the mean weight of the adrenal glands between animals of groups I and III ($P = 0{\cdot}7$–$0{\cdot}6$); but the mean weight of the glands of the animals of group II was significantly lower than that of either groups I or III ($P < 0{\cdot}01$). The mean weight of the right adrenal glands was 3·3–4·5 mg. greater than that of the left glands in each of the three groups of ferrets ($P < 0{\cdot}01$). The mean ovarian weight was considerably lower in animals of group I than in animals of groups II and III, that of the latter group being the greatest. The mean body weight was approximately the same in animals of groups II and III, but was lower in animals of group I.

HISTOLOGY

The glands are enclosed in a thick outer connective tissue capsule of rather coarse collagenous fibres, which contains blood vessels and nerves. Trabeculae of connective tissue extend from the deeper capsular layers, and accompany blood vessels which penetrate into the glandular parenchyma.

Cortex. The three main zones characteristic of the mammalian gland, the z. glomerulosa, z. fasciculata and z. reticularis, were readily distinguishable in all specimens (Pl. 1, fig. 1).

The z. glomerulosa is always well developed, and consists of single or double columns of cells which form loops immediately beneath the outer capsule. The peripheral arcs of cells and the columns themselves are often separated by the prominent trabeculae of connective tissue mentioned above. The cells vary considerably in shape, but they are often somewhat flattened, and each contains a single prominent round nucleus. The whole zone usually stains lightly by contrast with the deeper layers of the cortex (Pl. 1, fig. 2).

Between the z. glomerulosa and the z. fasciculata lies a narrow irregular band of cells forming the z. intermedia (Pl. 1, fig. 2). The cells here are smaller than those in either of the adjacent zones, and usually appear compressed or irregular in outline; in many places the cell boundaries are indistinct, and the zone is conspicuous chiefly on account of the closely packed nuclei.

There is usually a gradual transition from the z. intermedia to the z. fasciculata. In the ferret this latter zone can be subdivided into outer and inner parts, although there is no well-defined boundary between the two. The cells of the outer part are the largest in the cortex, quadrangular or polyhedral in shape, and contain a single centrally placed nucleus surrounded by abundant cytoplasm which in paraffin-embedded material often appears vacuolated or spongy. The cells are usually arranged in columns extending towards the medulla, but in some areas of the sections the columnar arrangement is poorly developed. Blood vessels extend between the columns, accompanied by delicate trabeculae of connective tissue, which are much less prominent than those in the z. glomerulosa. In the deeper layers of the z. fasciculata the cells are smaller and stain more deeply, and there is a gradual but irregular transition to the inner part of the zone. The columnar arrangement of cells is usually maintained, but is generally less clearly defined than in the outer layers.

The z. reticularis is extremely variable both in its prominence and in its cellular constitution. The cells of the zone are smaller than those of any other cortical layer except the z. intermedia. Some resemble small cells of the inner z. fasciculata; others have small irregular darkly stained nuclei surrounded by little cytoplasm, and many resemble small lymphocytes in appearance (Pl. 1, fig. 3). The arrangement of the cells is irregular; in some glands they are closely packed together, but in others they are separated by abundant connective tissue which is always more prominent in this zone than in the z. fasciculata, and often appears to be arranged circumferentially around the central medulla. Large thin-walled blood vessels are usually prominent in the z. reticularis.

In some areas cells of the inner z. fasciculata appear to extend up to the cortico-

medullary junction without the interposition of a typical z. reticularis, but this arrangement is variable even in different areas of a single section. Connective tissue at the cortico-medullary boundary is usually scanty, and a distinct medullary connective tissue capsule has never been observed.

A few cells which show all the morphological characteristics of cells of the inner z. fasciculata lie between some parts of the z. reticularis and the medulla. These cells often form small groups, or extend in short lines one or two cells in thickness (Pl. 1, fig. 4). They have been assumed to constitute a z. juxtamedullaris.

A further notable feature of the glands is the constant occurrence of islands of cortical cells lying amidst the cells of the medulla. Many of these islands can be traced into continuity with juxta-medullary cells lying internal to the z. reticularis, but some appear to be completely isolated from cortical tissue. All, however, resemble cells of the inner z. fasciculata.

It was not possible to correlate structural features of the glands with the stage of oestrus. Furthermore, the few glands from male animals which were examined showed no significant differences from those of the female animals.

Medulla

In formalin-fixed material the cells of the medulla appear large and pale-staining, with granular cytoplasm surrounding a single large round nucleus (Pl. 1, fig. 3). The cells are usually elongated, and lie in double rows or islands between capillaries and thin-walled sinusoids. The cells show a typical chromaffin reaction after fixation in formol-dichromate. Groups of large ganglion cells were occasionally observed lying either in the medulla or at the cortico-medullary border.

HISTOCHEMICAL OBSERVATIONS

Lipid

In anoestrous animals the cells of the z. glomerulosa contain abundant lipid which colours strongly with Oil Red O (Pl. 2, fig. 5). The droplets are usually large and often fill the whole cytoplasm of the cells. Internal to the z. glomerulosa is a band of variable width in which lipid is either absent, or present as scattered fine droplets. This 'lipid-poor' zone corresponds roughly to the z. intermedia. In the z. fasciculata lipid is usually most abundant in cells of the outer half or two-thirds of the zone, where the intensity of staining often equals that of the z. glomerulosa, although the droplets are usually smaller in the z. fasciculata. Irregular staining of the z. fasciculata often results from the fact that some columns of cells contain more stainable lipid than neighbouring ones (Pl. 2, fig. 5).

Cells nearer the z. reticularis contain less lipid, and cells of the z. reticularis itself are either devoid of lipid, or contain only a few small droplets. Juxta-medullary cells resemble cells of the inner z. fasciculata in their content of lipid; and the islands of cortical cells lying in the medulla invariably contain numerous lipid droplets and stain strongly.

An increase in the amount of cortical lipid was found in glands taken from some of the animals killed in late pro-oestrus or full oestrus. This was most apparent in the inner layers, namely, the inner z. fasciculata and the z. reticularis. These inner

layers usually still stained less strongly than the peripheral ones, but cells rich in lipid extended to the medullary border (Pl. 2, fig. 6). In some of these glands the z. intermedia was indistinct, and its cells contained as much lipid as cells of the outer z. fasciculata; in others, the z. intermedia remained relatively free from lipid.

Glands from other ferrets killed during full oestrus, however, resembled those from anoestrous animals, and there was also considerable variation in the appearance of glands from ferrets killed in pro-oestrus. Thus it was not usually possible to correlate the oestrous state of an animal with the pattern of lipid in the adrenal cortex.

Non-specific esterase

A positive reaction for non-specific esterase occurs throughout the whole cortex (Pl. 2, fig. 7). The reaction is usually weakest in cells of the z. intermedia, and strongest in the inner z. reticularis and intramedullary islands of cortical cells. Medullary cells give a slight positive reaction in their peri-nuclear region. There was possibly some intensification of the reaction in the inner half of the cortex in glands taken from animals in late pro-oestrus and full oestrus.

Alkaline phosphatase

A strong positive reaction for alkaline phosphatase is found in the outer fibrous capsule of the glands, and in the trabeculae of connective tissue extending inwards from it (Pl. 2, fig. 8). The cytoplasm of the cells of the outer two layers of the cortex is only doubtfully positive, although an intense reaction occurs in the nuclei of these layers. Both nuclei and cytoplasm of the cells of the z. reticularis appear positive, but the reaction in the connective tissue of this zone is strong, and some diffusion artifact may occur. The medullary cells are negative, but outlined by a strong positive reaction in the blood vessels and stroma. Intramedullary cortical tissue shows a similar reaction to cells of the inner z. fasciculata.

Acid phosphatase

A positive reaction for acid phosphatase occurs in the nuclei of the whole cortex, although this is less marked in cells of the z. glomerulosa. The cytoplasm of some cells in the middle of the z. fasciculata contain positive granules. The cytoplasm of medullary cells is diffusely positive (Pl. 3, fig. 9), and often contains a group of strongly positive peri-nuclear granules.

Cholinesterase

True and pseudo cholinesterase can be demonstrated in nerve fibres in the capsule, and in some sections positive fibres can be traced inwards towards the medulla. The capsule itself is negative. The cells of the z. glomerulosa are usually negative, while the z. fasciculata and z. reticularis are patchily positive for pseudo cholinesterase. The medullary cells are negative, but outlined by strongly positive nerve fibres, connective tissue and blood vessels.

Animals killed during late pro-oestrus or full oestrus failed to show any gross or constant variation in the distribution or intensity of activity of alkaline and acid phosphatase or cholinesterase.

ACCESSORY ADRENAL TISSUE

One or more detached nodules of accessory adrenal tissue were found in 11 of 135 ferrets examined during the past year; the actual incidence may, however, be greater than this figure suggests, since small nodules may be embedded in peri-renal fat and thus escape observation. The largest mass of tissue, which was situated on the ventral aspect of the right renal artery, measured 2·8 mm. in length by 1·7 mm. in breadth and weighed 3·2 mg. Other nodules weighed between 0·4 and 3·0 mg.

These accessory bodies consisted only of cortical tissue, and contained all the typical layers, although they were less clearly differentiated from one another than those in the main cortex (Pl. 3, fig. 10). Some of the larger masses of accessory tissue were themselves nodulated, and divided to an incomplete extent by penetration of trabeculae from their connective tissue capsule. In one of the isolated nodules a central core of connective tissue enclosed pale-staining vacuolated cells, whose cytoplasm contained light brown pigment granules.

Nodules closely associated with the main glands were common. Many were partly embedded in the outer cortex, and projected as tubercles on the surface of the gland; others lay deeply in the cortex, or less commonly within the medulla. Nodules lying superficially were completely or partially enclosed within a connective tissue capsule (Pl. 3, fig. 11), and as in the case of detached accessory tissue, usually contained all the typical cortical layers in their usual order, although sometimes these were poorly defined. Nodules which lay deeply in the cortex, or which encroached on the medulla, were always associated with a trabeculum of connective tissue which penetrated the gland from the outer connective tissue capsule, and which was continuous with a central connective tissue core of the nodule (Pl. 3, fig. 12). In such specimens the layers of the nodule were reversed, so that the z. glomerulosa was situated centrally around the connective tissue core, and the z. reticularis enclosed the nodule peripherally. No medullary tissue was found in any of these nodules.

DISCUSSION

It is apparent from these studies that the blood supply of the adrenal glands of the ferret is both extensive and variable. Flint (1900) reached a similar conclusion from his studies on the adrenal glands of the dog; and Bennett & Kilham (1940), who studied the blood supply to the glands of the cat, found that in this animal there is considerable variation both in the number of arteries supplying the glands, and also in the origin of these vessels. These workers came to the conclusion that there is no single pattern of arterial supply which could be regarded as typical, a finding which also applies to other mammals (see Harrison & Hoey, 1960).

The anatomical relationships of the adrenal glands of the ferret suggest that total adrenalectomy would be a difficult operation to carry out in this animal. The major difficulty would be the removal of the right gland from the closely adherent posterior vena cava without causing serious haemorrhage. A second problem would be to ensure that all adrenal tissue had been removed, for although the larger accessory nodules are often readily visible, smaller ones may be concealed in fatty

tissue, so that a block dissection of the whole upper peri-renal area might be necessary to ensure a complete adrenalectomy.

Histologically the ferret's adrenals conform to the pattern found in the majority of mammals. Thus the cortical cells are arranged in three main zones, glomerulosa, fasciculata and reticularis. A z. intermedia, which was well defined in the majority of the ferrets studied, has also been described in the glands of other carnivores such as the dog and the cat (Nicander, 1952), as well as in many animals of other orders, such as the rat (Mitchell, 1948) and cow (Nicander, 1952).

A feature which appears to be less frequent in mammals is the z. juxtamedullaris, which consists of cortical cells of a type differing from those of the z. reticularis situated internal to this latter zone. Nicander (1952) reported that such a zone occurred in the adrenal glands of horses, rabbits and rats, but not in those of dogs and cats. Bennett, however (1940), noted islands of cortical cells lying within the medulla of cat adrenal glands, and since Nicander (1952) suggested that intramedullary cortical cells in the ox adrenal might constitute a juxtamedullary zone, there may be some justification for regarding intramedullary cells in the cat as the equivalent of juxtamedullary tissue.

In the ferret the small collections of cells resembling those of the inner z. fasciculata which lie internal to the z. reticularis appear to constitute a true but inconstant z. juxtamedullaris. Intramedullary cortical cells are a conspicuous feature in ferrets' adrenals, and since many of these islands of cells can be traced into continuity with juxtamedullary groups, it is probably logical to consider all such cells under the general term 'juxtamedullary'.

There is no doubt at the present time that adrenal function is closely correlated with sexual activity. Kolmer in 1918 noted an enlargement of the adrenal glands of the mole during the mating season, and Watson (1923), who studied the same animal, found that the size of the glands was greatest, and that they contained most lipid material during the breeding season in April. In the rat the glands have been found to be larger at oestrus than at dioestrus (Andersen & Kennedy, 1932; Bourne & Zuckerman, 1940) mainly due to an increase in size of the cortex, especially the z. fasciculata. Baker (1938) found that in dogs hypertrophy of the cortex and medulla occurred during oestrus, although only in a few of his groups of animals. In the cat, Lobban (1952) found that the ratio z. fasciculata: z. reticularis decreased in oestrus, pro-oestrus, pregnancy and pseudo-pregnancy, due to an increase in the width of the zona reticularis. Numbers of other investigators have noted similar findings (Bourne & Zuckerman, 1940; Chester Jones, 1957).

In the ferret, some evidence was found that the weight of adrenal tissue is lower in animals killed between January and March than in those killed later in the year during late pro-oestrus, oestrus or anoestrus. Histologically there did not appear to be any striking variation between the glands of any of the groups of animals; and although an increase in stainable cortical lipid was found in some late pro-oestrous and oestrous animals, this was not sufficiently constant to be considered as a criterion of adrenal function. In any event there is probably no close correlation between the lipid and hormone content of the adrenal cortex (Deane & Greep, 1946; Deane & Seligman, 1953), although there is no unanimity as to which histochemical tests are most suitable to demonstrate the functional state of the gland. Small

variations in enzyme activity cannot be readily assessed by histochemical methods, and the various enzyme studies applied in the present investigation failed to reveal any significant difference between the glands of sexually active and inactive animals.

The actual distribution of lipid in the cortex of the ferret's adrenal appears to resemble the pattern described by Nicander (1952) in the dog, but differs from that in the cat. The localization of acid and alkaline phosphatase in the ferret resembles that in the glands of both the cat and dog (Nicander, 1952), while the distribution of cholinesterase in the cortex is the reverse of that found in the cat (Coupland & Holmes, 1958), in which animal the z. glomerulosa is strongly positive for pseudo-cholinesterase, and the rest of the cortex negative.

In conclusion some reference must be made to the accessory nodules of cortical tissue. Detached nodules can certainly be considered 'accessory', and possibly nodules which are attached to a main gland, but completely separated from it by fibrous tissue, can be included in the same category. On the other hand, nodules whose cells are in continuity with the main cortex probably arise by ingrowth of capsular fibres into the gland during development as was suggested by Waring & Scott (1937). Zwemer, Wotton & Norkus (1938) also stressed the role of the capsule in determining cortical morphology, and both they and Bennett (1940) noted that intramedullary nodules in the cat showed reversal of cortical zones, so that the z. glomerulosa lay centrally around a core of connective tissue. The present study has shown that in the ferret the orientation of cortical layers relative to the capsule follows the typical pattern in nodules which are detached from the main glands, or which lie superficially within the cortex. The layers in nodules which lie deeply, on the other hand, are orientated with reference to the connective tissue which forms a core to the nodule. These findings suggest that in the ferret, as in other animals studied, the capsule may be important in determining the structure of the gland, but this suggestion requires some further evidence, which might be obtained from enucleation experiments such as those performed on the rat by Greep & Deane (1949) and Chester Jones & Spalding (1954).

SUMMARY

1. The anatomy, histology and some histochemical characteristics of the adrenal glands of mature ferrets have been studied.

2. The blood supply was found to be extensive and, as in other carnivores, extremely variable.

3. The weight of the glands was greater during oestrus and early anoestrus than during late anoestrus or early pro-oestrus. The right gland was heavier than the left.

4. The z. glomerulosa, z. intermedia and z. fasciculata were well developed. The z. reticularis was usually poorly developed, and variable in appearance. An irregular z. juxtamedullaris was present, and intramedullary islands of cortical cells were common.

5. The distribution of lipid, and alkaline and acid phosphatase activity resembled that described in the dog's adrenal.

6. Accessory adrenal tissue detached from the main glands was found in 11 of

135 animals. It consisted only of cortical tissue arranged in the same pattern as the main cortex.

7. Nodulation within the main cortex was common. Nodules which lay deeply showed an inversion of cortical zones around a central connective tissue core.

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EXPLANATION OF PLATES

C, capsule; ZG, z. glomerulosa; ZI, z. intermedia; ZF, z. fasciculata; ZR, z. reticularis; ZJM, z. juxtamedullaris; M, medulla; IC, inner cortex; CT, connective tissue.

Plate 1

Fig. 1. Section to show the main zones of the cortex. Masson's stain. ×110.

Fig. 2. Section through the outer cortex. Note the pale z. glomerulosa, the closely packed nuclei of the z. intermedia, and the transition between this zone and the z. fasciculata. Masson's stain. ×195.

Fig. 3. Small cells of the z. reticularis, with scanty cytoplasm. Masson's stain. ×195.

Fig. 4. Cells resembling those of the z. fasciculata lying between the z. reticularis and the medulla (z. juxtamedullaris). Cells of the z. reticularis in this section are larger than those shown in Fig. 3. Haematoxylin and eosin. ×410.

Plate 2

Fig. 5. Frozen section showing the typical distribution of lipid in the cortex. Oil Red O. ×80.

Fig. 6. Frozen section showing the distribution of lipid found in some oestrous ferrets. Oil Red O. ×80.

Fig. 7. Esterase reaction in the adrenal. Note clumps of cortical cells in the medulla. Frozen section, α-naphthyl acetate method. ×80.

Fig. 8. Alkaline phosphatase in the adrenal. Note the intramedullary island of cortical cells (I.S.). Frozen section, Gomori method. ×80.

Plate 3

Fig. 9. Acid phosphatase reaction. Nuclei alone stain in the inner cortex, but the medullary cells are diffusely positive. Frozen section, Gomori method. ×88.

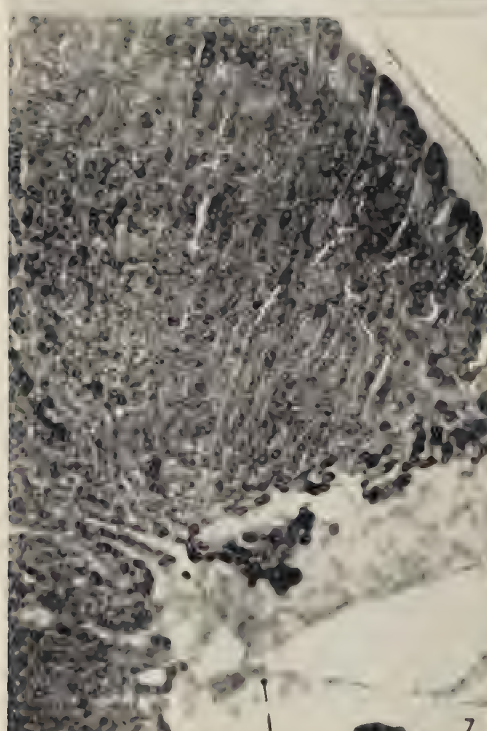
Fig. 10. Section through a detached nodule of accessory adrenal tissue. Masson's stain. ×88.

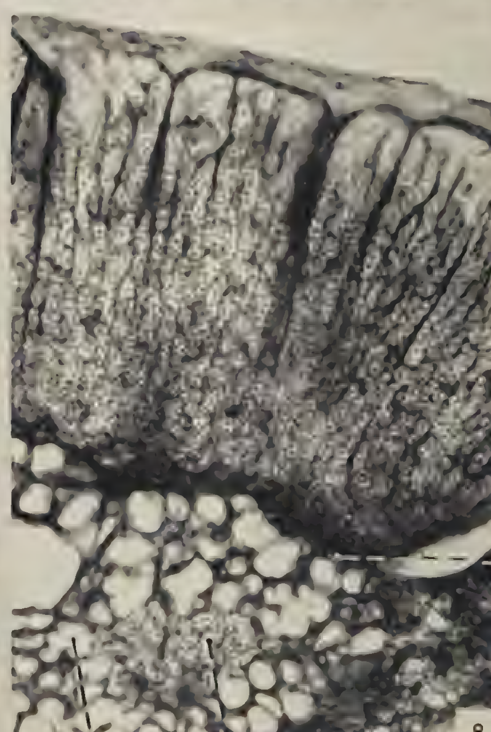
Fig. 11. A nodule of cortical tissue which is incompletely separated from the main cortex by capsular connective tissue. The z. glomerulosa of the main gland is indicated. Masson's stain. ×88.

Fig. 12. An intramedullary nodule of cortical tissue, showing inversion of cortical layers around a central core of connective tissue which penetrates from the capsule. The z. glomerulosa of the nodule and that of the main gland are in continuity. Haematoxylin and eosin. ×88.

R. L. HOLMES—THE ADRENAL GLANDS OF THE FERRET

(*Facing p.* 336)





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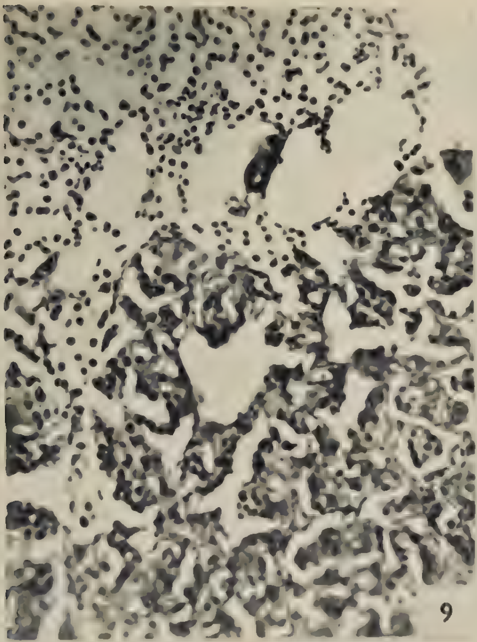

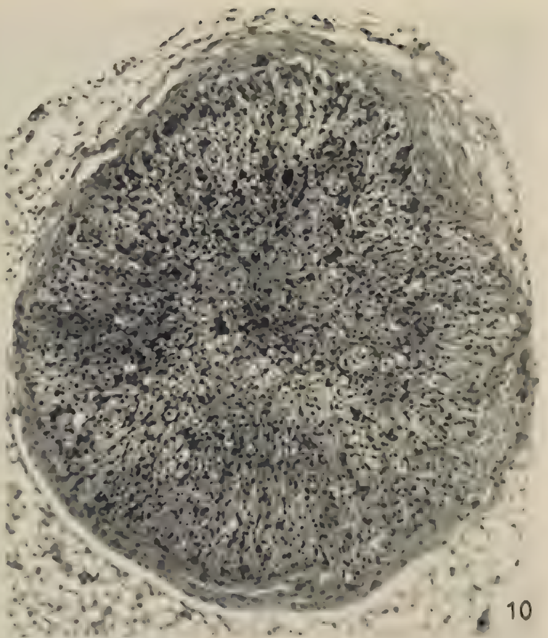

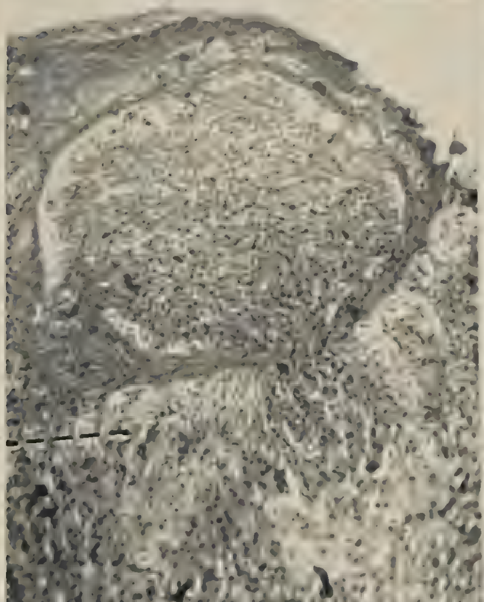

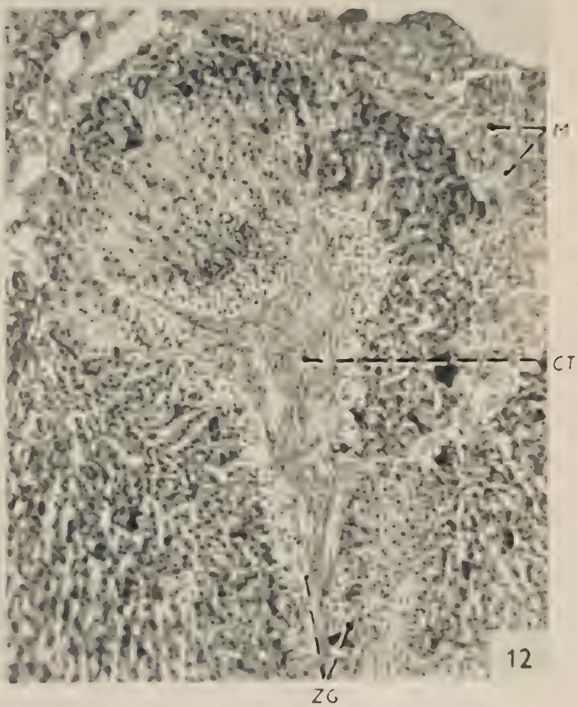

R. L. HOLMES—The adrenal glands of the ferret

HUMAN LOWER LUMBAR VERTEBRAE: SOME MECHANICAL AND OSTEOLOGICAL CONSIDERATIONS

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INTRODUCTION

In man, the vertebral column supports the weight of the trunk and upper limbs, and also sustains much of the weight of burdens borne upon these parts of the body. The structure of the chain of vertebral bodies and intervertebral discs (Wyman, 1857; Wagstaffe, 1874; Gallois & Japiot, 1925) and mechanical considerations (Rauber, 1876; Petter, 1933; Bradford & Spurling, 1945; Davis, 1959) indicate that these weights subject this chain mainly to vertical compression forces, the magnitude of which increase from the axis to the lumbo-sacral joint. Therefore one would expect the size of the vertebral bodies to increase in the same direction, the lowest lumbar vertebra being the largest. In general this assumption is supported by serial measurements of the vertebral bodies, the linear dimensions increasing down to the level of the third or fourth lumbar vertebra. In the last two lumbar vertebrae, however, some variation occurs, and in some cases the linear diameters of the vertebral bodies are decreased: for example, in Aeby's (1879) series, two out of twenty-eight columns had a smaller transverse diameter in L5 than in L4; in Cunningham's (1886) series of sagitto-vertical indices in fifty-seven columns from many races, twenty-two had a larger index for L5 as compared with L4, it being equal or smaller in the remaining thirty-five; Anderson (1883), giving average dimensions from a series of twenty-eight columns, found that the antero-posterior diameter of L4 was less than that of L3, and that on average this diameter of L5 equalled that of L3. However, since resistance to pressure by a uniform structure depends upon its cross-sectional area, linear dimensions alone might give a false impression. Vertebral bodies are fairly uniform in internal structure (Gallois & Japiot, 1925), and it is therefore to be expected that their cross-sectional areas, or more simply the areas of the upper or lower surfaces of the vertebral bodies, would better represent their ability to resist longitudinal compression than do their linear dimensions. Davis (1955, 1958) has measured the areas of the upper surfaces of the vertebral bodies in nineteen columns, and his findings support those of the previous authors, for in eight out of the nineteen the area of L4 was equal to or larger than that of L5.

Reduction in size of the lower one or two lumbar vertebral bodies suggests that less compressive force is exerted upon them than on those immediately above; this being so, part of the total compressive force must be transmitted to the pelvis by some other mechanism. The inaccessibility of the lumbar vertebrae and of the lumbo-sacral joint makes direct assessment of the forces acting in this region extremely difficult. If, however, one assumes that the size of a given portion of a vertebra is related to the magnitudes of the forces acting upon it, then comparison

of the size of that portion in different vertebrae from one individual should provide an assessment of the relative magnitudes of those forces at different levels.

In most subjects the normal lumbar lordosis present in the upright position places the disc surfaces of the lower lumbar vertebral bodies at an angle to the vertical (Fig. 1) so that the vertical compression from above (*A* in Fig. 1) can be resolved into two components, one acting obliquely downwards and backwards (*B*) the other acting forwards (*C*). Component *B* is clearly supported by the vertebral bodies; component *C*, which tends to slide the fifth lumbar vertebra forwards into the pelvis,

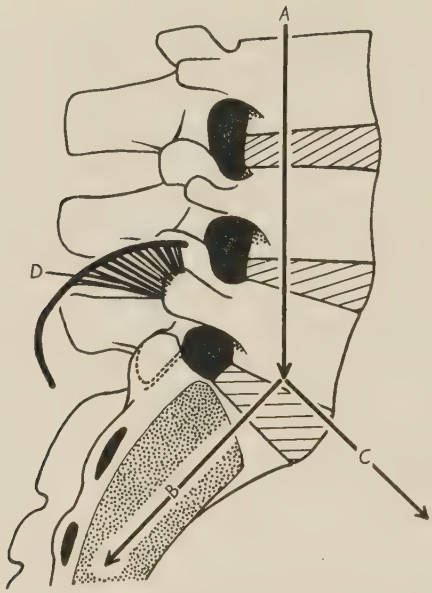

Fig. 1. Diagram to show the mechanical considerations. *A* is the vertical compression force, which can be divided into postero-inferior (*B*) and antero-inferior (*C*) components. *D* is the superimposed outline of the posterior part of the right iliac crest with the posterior part of the ilio-lumbar ligament attaching it to the sacrum. The plane of the lumbo-sacral zygopophyseal joint surfaces are shown in broken outline. (The right ala of the sacrum has been removed.)

would appear to be resisted by appendages of the fifth lumbar neural arch: the lumbar and lumbo-sacral zygopophyseal joint surfaces (broken line in Fig. 1) are so placed that normally they can prevent forward sliding of L 5 upon the sacrum, and as the ilio-lumbar ligaments pass obliquely forwards from ilium to transverse process they too may resist such a tendency. Since the fifth lumbar vertebra is exposed to both components, one might expect two structural modifications. First, an increase in relative size of its pedicles to transmit forces from the vertebral body to the neural arch, and secondly, should the ilio-lumbar ligaments be mainly responsible one would expect also an increase in relative size of its transverse processes.

For these reasons measurements have been made of the areas of the lower lumbar vertebral bodies and of the sizes of the pedicles and transverse processes in a series of late juvenile and adult West African vertebral columns, and the results collated.

MATERIAL

Through the kindness of Prof. Smith at Ibadan it was possible to examine his very large collection of West African material.

Forty-two male and thirty female West African vertebral columns were selected from over 200 adult and young adult columns available as being those which were free from artefacts, pathological changes and numerical and other anomalies. While the stated ages at death were available for most of these skeletons, little reliance could be placed upon them as births are not registered, and precise age plays little part in West African society; this series has therefore been divided into adult (35♂, 24♀) and young adult (7♂, 6♀) groups; those in which not all, but at least eight vertebrae have their epiphyseal rings fused to the bodies have been classified as young adults. A juvenile series of seven columns was also examined, including those in which the rings were present but had fused in less than eight vertebrae.

METHODS

The areas of the lower vertebral body surfaces have been obtained by tracing their outline on to paper with a sharp hard pencil, and then measuring the areas in square centimetres with an architectural planimeter.

The size of the pedicles was assessed by obtaining the pedicle index (Davis, 1955) which is the product of the greatest and least diameters of a pedicle at its most slender portion. These were measured with callipers. The mean of the indices of the two sides was then calculated to give the *mean pedicle index* for each vertebra.

The size of the transverse processes was assessed by obtaining the product of the greatest and least diameters at a point one-third of the distance from the lateral aspect of the superior articular process to the tip of the transverse process, measured with calipers. The buttress for the articular process terminates medial to this point and most of the muscular attachments lie laterally; the dimensions at this point are thus those best suited to mechanical analysis. The mean of the values of the two processes in each vertebra was then calculated, and called the *mean transversal index*.

Thus, three figures were obtained in each of the lower three lumbar vertebrae in each column, namely the area of the lower surface of the vertebral body, the mean pedicle index, and the mean transversal index.

In order to allow comparison of these indices in different individuals, in each column the ratios $(L3 \times 100)/L4$ and $(L4 \times 100)/L5$ were then calculated for each of these indices. In the adult and young adult series the ratios of the areas were compared with those derived from the pedicles, and then with those derived from the transverse processes, by graphic means and by regression calculations.

RESULTS

The measurements

(*a*) *The areas of the lower surfaces of the vertebral bodies* (Table 1)

In all groups the mean area of the lower surface of the body of L4 is slightly greater than that of L3. The mean area of L5 is smaller than that of L4. In both

Table 1. *The areas in square centimetres of the lower surfaces of the lower three lumbar vertebrae in seventy-two West Africans*

	Males				Females			
	Mean area (cm.²)	Range	Standard deviation	No. of columns	Mean area (cm.²)	Range	Standard deviation	No. of columns
				Adults				
L3	13·4	11·1–16·1	1·1	35	11·3	9·3–13·6	1·0	24
L4	14·0	11·6–17·0	1·2		11·9	9·6–13·8	1·0	
L5	12·8	10·2–16·4	1·3		11·3	8·8–12·5	1·0	
				Young adults				
L3	12·9	10·4–15·0	1·5	7	11·0	9·0–14·8	2·1	6
L4	13·3	10·7–16·0	1·6		11·9	9·9–16·3	2·2	
L5	12·2	10·2–14·7	1·4		11·1	9·0–14·9	2·0	

male groups the mean area of L5 is smaller than that of L3 also. The findings in the two sexes do not differ significantly from each other.

Summarizing the results for individual columns the area of L3 was greater than that of L4 in five out of forty-two males, and one out of thirty females; that of L4 was greater than that of L5 in thirty-five out of forty-two males and twenty-five out of thirty females: the differences in frequency in the two sexes is not significant in either case.

Table 2. *The mean pedicle indices in square centimetres in adult and young adult West Africans*

	Males				Females			
	Mean area (cm.²)	Range	Standard deviation	No. of columns	Mean area (cm.²)	Range	Standard deviation	No. of columns
				Adults				
L3	1·40	0·89–1·73	0·18	35	1·23	0·88–1·62	0·21	24
L4	1·61	1·10–2·10	0·25		1·37	1·02–1·83	0·26	
L5	2·17	1·44–3·23	0·42		1·90	1·35–2·58	0·31	
				Young adults				
L3	1·32	1·00–1·84	0·28	7	1·12	0·78–1·59	0·29	6
L4	1·59	1·23–2·34	0·36		1·32	0·94–1·75	0·29	
L5	2·01	1·49–2·64	0·40		1·68	1·50–2·08	0·21	

(*b*) *The mean pedicle indices* (Table 2)

The mean pedicle indices have a constant pattern in all groups, there being an increase from L3 to L5. The magnitudes of the standard deviations indicate the considerable variability in individual measurements, this being most marked in the fifth lumbar vertebra. Again the findings in the two sexes did not differ significantly.

(*c*) *The mean transversal indices* (Table 3)

The mean transversal indices show that the fifth lumbar transverse processes are much stouter than their higher counterparts, and that on average the fourth lumbar

Table 3. *The mean transversal indices in square centimetres in adult and young adult West Africans*

	Males				Females			
	Mean area (cm.²)	Range	Standard deviation	No. of columns	Mean area (cm.²)	Range	Standard deviation	No. of columns
				Adults				
L3	0·36	0·24–0·50	0·07		0·29	0·20–0·56	0·08	
L4	0·26	0·14–0·55	0·09	35	0·26	0·15–0·50	0·09	24
L5	1·20	0·52–2·87	0·53		0·78	0·25–1·41	0·99	
				Young adults				
L3	0·32	0·17–0·50	0·10		0·26	0·19–0·32	0·05	
L4	0·25	0·20–0·30	0·04	7	0·19	0·14–0·28	0·05	6
L5	0·87	0·45–1·55	0·41		0·77	0·19–1·54	0·42	

process is less stout than the third in all the four groups. As with the pedicle indices, the greatest individual variation occurs in the fifth lumbar vertebra.

(*d*) *Juveniles* (Table 4)

Although the differences are not so striking in these young specimens, they do have an over-all pattern similar to that of the adults, with the exceptions of the transversal indices in J3 and J4, in which the fifth lumbar transverse processes are less robust than in those above.

Table 4. *The measurements in five male and two female juvenile West African columns*

		Male columns					Female columns	
		J1	J2	J3	J4	J5	J6	J7
Stated age (years) ...		6	7	9	9	10	8	12
Vertebral body area (cm.²)	L3	7·3	7·9	9·0	9·2	9·6	8·5	6·3
	L4	7·4	8·9	9·5	10·3	10·2	9·2	6·4
	L5	7·0	9·2	9·3	9·7	9·4	9·0	6·2
Mean pedicle index (cm.²)	L3	1·20	0·99	0·86	1·03	0·94	0·65	0·60
	L4	1·19	1·29	0·90	1·10	1·05	0·85	0·61
	L5	1·26	1·30	0·98	1·19	1·23	1·24	0·87
Mean transversal index (cm.²)	L3	0·37	0·35	0·25	0·28	0·27	0·22	0·22
	L4	0·22	0·35	0·21	0·15	0·20	0·20	0·20
	L5	0·42	0·36	0·21	0·13	0·72	0·64	0·35

One can summarize these results by saying that most frequently the area of the lower surface of the body of L4 is larger than in either L3 or L5, that the pedicles increase in size from above downwards, and that the transverse process of L5 is usually much larger than that of L3 or L4, L3 being larger than L4.

The ratios

(1) Comparisons between the relative sizes of the vertebral pedicles and the areas of the bodies are presented graphically in Figs. 2 and 3.

It will be noted that in Fig. 2, which compares the ratios $(L3 \times 100)/L4$ for pedicles and areas, there is no clear correlation between the differences in the areas

and those in the pedicles in these two vertebrae in any group. Fig. 3, which compares the ratios (L4 × 100)/L5 for pedicles and vertebral body areas, shows an apparent inverse correlation between the ratios, that is to say that the smaller the area of L5 in relation to L4, the bigger the size of the L5 pedicle relative to L4. Statistical tests of this show that this correlation is significant. Comparing the two sets of figures in both males and females, one obtains for males $r = -0{\cdot}633$, $t = 10{\cdot}00$, $P < 0{\cdot}001$; for females $r = -0{\cdot}798$, $t = 11{\cdot}62$, $P < 0{\cdot}001$. The correlation does not differ significantly between the two groups ($z♂ - z♀ = 0{\cdot}353$, s.e. $= 0{\cdot}248$). Regression lines for the male and female groups have been inserted in the figure.

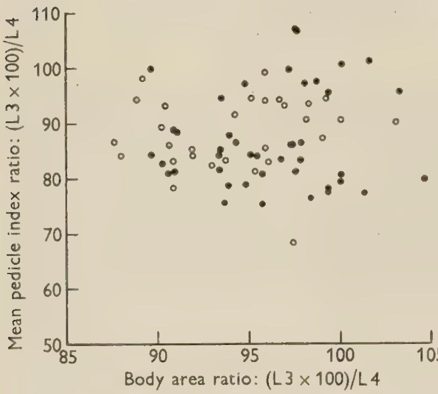

Fig. 2

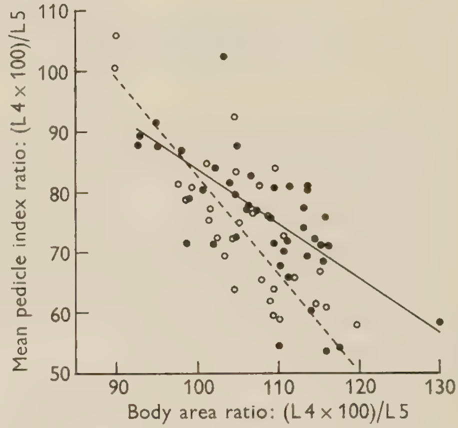

Fig. 3

Fig. 2. Comparison of the mean pedicle index ratios and body area ratios (L3 × 100)/L4 in West Africans. (For description see text.) Males, ●; females, ○.

Fig. 3. Comparison of the mean pedicle index ratios and body area ratios (L4 × 100)/L5 in West Africans. (For description see text.) Males, ●; females, ○. Male regression line, ——; female regression line, - - - -.

This significant negative correlation shows that, on average, the smaller the relative area of the vertebral body of L5, the greater is the thickness of its pedicle.

(2) Comparisons of the mean transversal index ratios (L3 × 100)/L4 with the area ratios (L3 × 100)/L4 are given in Figs. 4 and 5.

As with the pedicle indices, there is no significant relationship between the changes in size of the 3rd and 4th lumbar vertebral body areas and those of the transverse processes (Fig. 4).

The (L4 × 100)/L5 ratios, shown in Fig. 5, suggest that when the area of L5 is small relative to that of L4, the thickness of the L5 transverse process is relatively large. Again, statistical treatment of the data gives significant results; for males $r = -0{\cdot}549$, $t = 5{\cdot}17$, $P < 0{\cdot}001$; for females $r = -0{\cdot}449$, $t = 2{\cdot}658$, $P < 0{\cdot}002$. The correlations in the two sets of figures do not differ significantly from each other ($z♂ - z♀ = 0{\cdot}134$, s.e. $= 0{\cdot}250$).

(3) The results therefore show that, in both sexes, the relative sizes of the pedicles and transverse processes of L3 and L4 are not dependent on the relative sizes of

their vertebral body areas; there is, on the other hand, a significant inverse relationship between the relative sizes of both the pedicles and the transverse processes of L4 and L5 when compared with the relative sizes of their vertebral body areas.

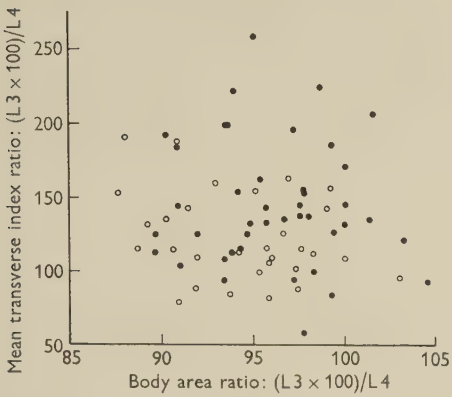

Fig. 4

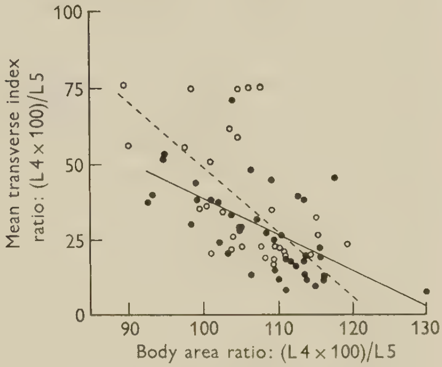

Fig. 5

Fig. 4. Comparison of the mean transverse index ratios and body area ratios (L3 × 100)/L4 in West Africans. (For description see text.) Males, ●; females, ○.

Fig. 5. Comparison of the mean transverse index ratios and body area ratios (L4 × 100)/L5 in West Africans. (For description see text.) Males, ●; females, ○. Male regression line, ——; female regression line, - - - -.

DISCUSSION

In this series of West African skeletons the finding that the area of the vertebral body of L5 is less than that of L4 in sixty out of seventy-four adult and young adult columns confirms the findings in other smaller series from different races, and suggests strongly that this is the common condition in man as a whole.

The significant inverse relationship between pedicle size and area of L5 when compared with L4 supports the view that the neural arch is responsible for transmission of part of the vertical compressive force from the vertebral column to the pelvis, and the similar relationship in size of the transverse processes suggests that part of this neural arch transmission is carried through the transverse process and, by inference, through the ilio-lumbar ligaments.

In most subjects it would appear that, in the upright position, pressure on the lumbo-sacral intervertebral disc is less than on the one above, the pressure on L5 being in part resisted by the neural arch. The neural arch components may be resisted by either the lumbo-sacral zygopophyseal joint surface or by the ilio-lumbar ligaments, and the significant inverse relationship between body size and thickness of transverse process in L5 suggests that these ligaments play a considerable part. Brailsford (1929) found that some 12% of 3000 subjects had lumbo-sacral zygopophyseal facets which faced inwards and could not therefore resist such a forward force; it would appear that in such individuals the ilio-lumbar ligaments are the sole mechanism resisting this tendency.

The lumbo-sacral intervertebral disc is the one most frequently affected by lumbar disc lesions (O'Connel, 1951; Armstrong, 1958). The present findings suggest

strongly that in the upright position the pressure on the lumbo-sacral disc is less than on those above; clinical findings (Armstrong, 1958) show that lumbar disc lesions are most commonly sustained with the spine flexed. In this flexed position the lumbo-sacral angle is reduced so that the anterior component of the compression force is of less importance, and there is an increase in magnitude of the vertical component. This increase in the vertical component, coupled with the relatively smaller cross-sectional area of the vertebral body surface (and hence of the disc) may well play an important part in prejudicing the integrity of the lumbo-sacral disc in the flexed position, particularly when it is remembered that Virgin (1951) has shown that the upper lumbar discs can sustain a greater compression force per unit area than can the lower ones.

I wish to record my gratitude to Prof. A. Smith of University College, Ibadan, for allowing me access to his magnificent skeletal material. My thanks are due to Dr and Mrs A. J. Palfrey for their liberal hospitality, assistance and advice, to Prof. R. E. M. Bowden for her constant encouragement, and to the staff of the Photographic Department of the Royal Free Hospital School of Medicine. The work was made possible by generous financial assistance from the Royal Society and Nuffield Foundation.

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THE GRANULE CELLS, MOSSY SYNAPSES AND PURKINJE SPINE SYNAPSES OF THE CEREBELLUM: LIGHT AND ELECTRON MICROSCOPE OBSERVATIONS

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INTRODUCTION

One of the special problems involved in electron microscopy of the central nervous system is the correlation of observations with those of light microscopy. In this respect the cerebellar cortex has certain advantages for it is everywhere arranged in three distinct layers, whose components are easily recognized with the electron microscope. Accounts by electron microscopy of limited aspects of this grey matter have been given by several workers (Fernández-Morán, 1957; de Robertis, 1959; Hager, 1959; Hager & Hirschberger, 1960; Palay, 1958). Among the many descriptions by light microscopy are those of Golgi (1894), Cajal (1911, 1926, 1954), Estable (1923), Carrea, Reissig & Mettler (1947) and more recently by Scheibel & Scheibel (1954), Fox & Barnard (1957), Jansen & Brodal (1958) Fox (1959), and Szentágothai & Rajkovits (1959).

Unfortunately so far, few attempts have been made to correlate directly the actual structure of the nervous system observed by the various methods of light microscopy, with those seen by the electron microscope. The Nissl picture of perikarya has been compared with structures observed by electron microscopy (see Palay & Palade, 1955). Also it is fairly well established that by light microscopy the neurofibrillar stains reveal axons and their terminals, but only when they contain neurofilaments (fine protein fibrils 100 Å. thick seen by electron microscopy in osmium-fixed preparations). Since, however, many axons and their terminals contain no neurofilaments, they may remain invisible by the silver method (see Palay & Palade, 1955; Boycott, Gray & Guillery, 1960, 1961). The relationship between the morphology of neurons revealed by the Golgi method and that seen by electron microscopy has been little investigated. In the cerebral cortex there is a close correlation however. The fine spinous processes of dendrites, thought by many to be Golgi-method artefacts, are clearly observed with the electron microscope, and are specialized sites of synaptic contact. Also the beading of axons and dendrites seen in Golgi and other preparations can be observed by electron microscopy in the form of dilatations in these processes (Gray, 1959*b*, *c*, 1961*a*).

The main purpose of this investigation is to compare observations with those made recently on the cerebral cortex (Gray, 1959*a–c*, 1961*a*) and to obtain answers to the following questions: Are the synaptic vesicles, membrane thickenings, mitochondria, etc., organized in the same way in the synapses of the cerebral and cerebellar cortices? Are the dendritic spines (well known light microscopic structures of the Purkinje cells) also sites of synaptic contact and is the spine apparatus present? What are the dimensions and nature of the extracellular spaces? Are the dendrites

and perikarya organized in the same way in the cerebrum and cerebellum, especially since the granule cells of the cerebellum appear as atypical neurons by light microscopy, because of the absence of Nissl material?

MATERIALS AND METHODS

Light microscopy

Sections of rat cerebellar cortex were examined using the conventional stains for nervous tissue. They involved (*a*) a Nissl stain to show the locations of the perikarya and proximal regions of their dendrites; (*b*) a Golgi-Cox method which selectively stains perhaps one in seventy neurons (Sholl, unpublished), it gives a more or less complete impregnation of a perikaryon and its dendritic ramifications and (*c*) a Cajal block silver stain to show the course of the axons. No light micrographs have been included in this paper for the cerebellar cortex has been described adequately using these methods by Cajal (1911, 1926, 1954), Scheibel & Scheibel (1954), Fox & Barnard (1957) and Fox (1959).

Electron microscopy

Adult rats were anaesthetized with ether and the skull roof and dura were removed to expose the cerebellum. Lobules VI and VII (Larsell, 1952) (the declive and tuber of the vermis) were removed in one piece and placed in 1% osmium tetroxide in mammalian Ringer, buffered at pH 7·4 and maintained at about 4° C. The lobes were cut into thin slices (0·5 mm. or less thick) and placed into fresh fixative. Fixation was continued for 3–4 hr. at about 4° C. with continuous gentle agitation. Afterwards the pieces were rinsed in distilled water, dehydrated in ethanol and then immersed in absolute ethanol containing 1% phosphotungstic acid for 3 hr., again with continuous agitation. The slices were finally embedded in Araldite for sectioning (see Gray, 1959*c*, 1961*b* for further details).

RESULTS

General organization of the cerebellar cortex observed by light microscopy

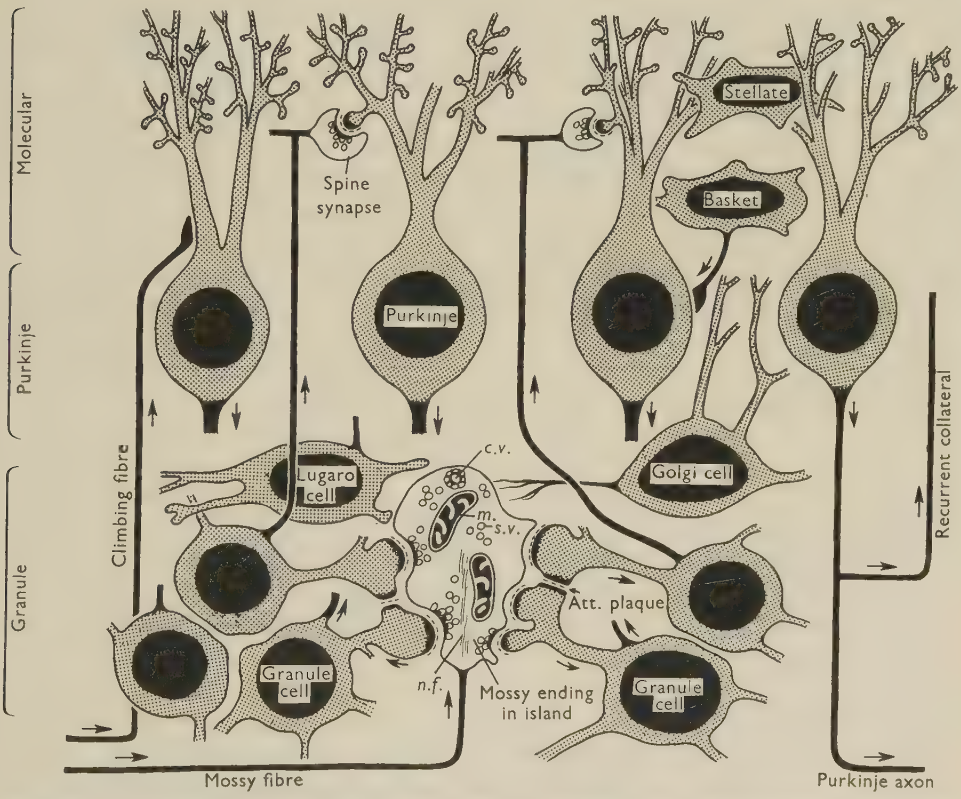
The cerebellar cortex has three layers, a deep layer containing numerous granule cells, a middle zone consisting of a single layer of Purkinje cell bodies and a superficial or molecular layer containing the ramifications of the Purkinje cell dendrites (Text-fig. 1). The climbing fibres and mossy fibres form the afferent supply to the cerebellar cortex. The latter form large endings situated in islands (glomeruli) between groups of granule cell bodies. Here they synapse with the tips of dendrites projecting towards them from neighbouring granule cell bodies. The axons of the granule cells run vertically into the molecular layer, where they branch and mingle with the spines of the branchlets of the Purkinje cell dendrites. Synaptic contacts cannot be observed by light microscopy. The axons of the Purkinje cells are the efferents of the cerebellar cortex.

All these features have long ago been described in detail by Golgi (1894), Cajal (1911), Estable (1923) and others, so micrographs are not included here. Details of the connexions of the climbing fibres, recurrent collaterals and smaller cerebellar neurons have been described by several workers and are mentioned in the Discussion.

The granule neurons

Light microscopy

The granule cell bodies (Text-fig. 1) form a layer that extends from the subcortical white matter to the Purkinje cell layer. Its thickness varies, being only about 30 μ in the troughs but exceeding 200 μ in the convexities of the folia. In Golgi preparations up to six dendrites can be observed arising from the granule cell perikaryon. They radiate out and terminate in short digit-like branches that contact the mossy fibre endings (see below). The axons arise either from the perikaryon or from one of the dendrites.



Text-fig. 1. Diagram of the cerebellar cortex based on light and electron microscopy (see text for details). Axons are shown as thick lines and the neuronal and dendritic cytoplasm are stippled. The connexions of the Golgi, Lugaro, basket and stellate neurons are not shown in detail. att. plaque, attachment plaque; *c.v.*, complex vesicle; *m.*, mitochondrion; *n.f.*, neurofilaments; *s.v.*, synaptic vesicles.

Electron microscopy

The granule cell nuclei (Pl. 1, fig. 1; Pl. 2, fig. 2; Pl. 4, fig. 6) are usually round or oval in shape and about 5 μ in diameter. The chromatin granules form clumps that are scattered through the nucleoplasm and are aggregated against the nuclear membrane. The cytoplasm is extremely sparse, being often only about 0·2–0·3 μ in diameter. It may decrease to as little as 200–300 Å.

Mitochondria occur in the cytoplasm, and where this is narrow they appear sandwiched between nuclear and surface membranes. The RNP granules (Pl. 1, fig. 1; Pl. 4, fig. 6) form small clusters that are scattered throughout the cytoplasm. Few membrane profiles of the endoplasmic reticulum (*m.e.*) are present and they show no special relationship with the majority of granules, in contrast to the granular reticulum complexes that have been described in certain other neurons (Palay & Palade, 1955).

Dendrites can occasionally be seen arising from the cell bodies (Pl. 3, fig. 5). They contain the characteristic tubules, each about 200 Å. in diameter (Palay, 1956; Gray, 1959*c*). Very occasionally similar tubules can also be observed in the cytoplasm of the perikaryon. The origins of the axons have not yet been identified with certainty by electron microscopy. They remain intracortical and are probably never myelinated (see below).

The granule cell bodies lie in groups and are in close contact with each other (Pl. 1, fig. 1) (Hager & Hirschberger, 1960). Their surface membranes lie within 200 Å. of each other over a considerable proportion of their apposed surface areas and in these regions no neuronal or glial processes intervene. It is only the outer neurones of a cluster that have regions of their surface membranes (Pl. 1, fig. 1, x) related to glial and neuronal processes of the neuropil. No axosomatic synaptic contacts have yet been observed either on these perikarya or the basal regions of their dendrites.

Occasionally dense staining cells (Pl. 1, fig. 1, *d.c.*) are observed in the granule layer. These are possibly neurons (see below) or microglia.

The mossy fibre endings

Light microscopy

There are apparently only two afferent supplies to the cerebellar cortex (Text-fig. 1), the climbing fibres running to the molecular layer and the mossy fibre endings terminating in the islands (glomeruli) of the granule layer (see above). In Nissl preparations these islands are seen as faintly basophilic masses lying between the groups of granule perikarya.

Electron microscopy

These islands are easily recognizable with the electron microscope. (Pl. 2, fig. 2; Pl. 5, fig. 8). Here the mossy fibre endings can be seen as large presynaptic bags up to 5μ in diameter. They contain masses of synaptic vesicles (about 500Å. in diameter), numerous mitochondria and sometimes neurofilaments. The bag has an irregular outline (compare Cajal, 1911) and is surrounded by smaller round or elongated pale profiles, many of which are sections of the branched tips of the granule cell dendrites that make synaptic contact with the mossy endings. Membrane thickenings occur at the contact regions. These various features will be considered in detail below.

These presynaptic bags can be identified as the mossy endings: (a) because of their position in the granule layer, (b) because of their large size, and (c) because they can occasionally be seen to originate from a myelinated axon (Pl. 3, fig. 3). There are other axon terminals in the island but these are small and are wholly unmyelinated (Cajal, 1911).

A single mossy fibre ending (Text-fig. 1) is shown at high magnification in Pl. 5, fig. 8. Unlabelled arrows indicate its surface membrane. At least five sorts of organelles are contained in the cytoplasm of these large presynaptic bags: (1) numerous mitochondria (*m.*) (2) synaptic vesicles (*s.v.*) 300–500 Å. in diameter; (3) complex vesicles (*c.v.*); (4) occasional large 'vesicles' (*ves.*) up to 2000 Å. in diameter; and (5) occasional bundles of neurofilaments (see below). In addition, a few tubules (200 Å, in diameter) have been seen running into the ending from the axon. Some endings appear very dense and are literally packed with synaptic vesicles (Pl. 3, fig. 4).

The complex vesicles are shown at higher magnification in Pl. 6, figs. 12, 13 and 15. They are tentatively interpreted as having a central sphere (600–800 Å. in diameter) surrounded by a shell formed from a closely packed layer of small vesicular bodies (each 150–200 Å. in diameter). These complex vesicles usually appear circular in sections, although elongated forms (suggesting division?) can occasionally be observed (Pl. 6, fig. 13, $c.v._1$). Some micrographs suggest that the complex vesicles arise from or fuse with the surface membrane (Pl. 5, fig. 8, $c.v._2$). Also they have been observed within a 'membranous' capsule (Pl. 6, fig. 15) together with synaptic vesicles. At present practically nothing is known about these complex vesicles. They have been observed by the author also in presynaptic processes of the rat cerebral cortex, lizard brain, rat and cat spinal cord and occasionally near the agranular reticulum of neuronal perikarya.

Smaller processes presumed to be sections of the branching dendritic terminals of the granule neurons surround and 'contact' the mossy fibre presynaptic bags. The dendrite tips contain tubules (200 Å. in diameter), mitochondria and one or two large vesicles (up to 2000 Å in diameter).

In these islands (glomeruli) two sorts of membrane thickenings can be observed (Pl. 5, fig. 8); (*a*) Symmetrical thickenings (*a.p.*), where the membranes of the dendritic processes are mutually apposed. These are tentatively designated dendro-dendritic attachment plaques. (*b*) Asymmetrical thickenings occur where the membranes of mossy endings and dendritic processes are apposed. The synaptic vesicles form aggregations on the presynaptic side of these thickenings.

Thickenings of the apposed axo-dendritic membranes. These are shown at three places in Pl. 5, fig. 8 (*m.t.*) and at higher magnification in Pl. 6, figs. 9 and 14 (Text-fig. 1). The thickening of the dendritic membrane (*p.m.t.*) is especially wide and dense. The axonal membrane thickening has groups of synaptic vesicles associated with it and some of those nearest the membrane ($s.v._1$) appear as vague dense bodies. However, another interpretation is that these bodies may be something other than dense-staining vesicles. The synaptic cleft is about 300 Å. across in contrast to the 200 Å. gap common between non-synapsing processes (see Gray, 1959*c*). A layer of material (*ma.*) lies in the synaptic cleft. This is either situated centrally or nearer to the postsynaptic membrane (see Gray, 1959*c*).

Thickenings of the apposed dendro-dendritic membranes. In the cerebellar islands (Text-fig. 1) thickened regions of apposed dendritic membranes (*a.p.*) are shown in several places in Pl. 5, fig. 8, and at higher magnification in Pl. 6, fig. 10 and 11. The membrane thickenings, in contrast to the axo-dendritic thickenings, appear quite symmetrical and tonofibrils (*ton.*) are sometimes present. In Fig. 10 there is a single

layer of material (*ma.*) in the cleft of the plaque and in fig. 11 two layers occur. So far these symmetrical thickenings have been observed on the apposed membranes of processes that are probably mainly dendritic although some might be glial. Axo-dendritic plaques with symmetrical structures have not so far been observed but many more observations are needed to decide this point. At present it is not possible to say whether the plaques occur between the dendritic branches of the same or different neurons. In the past attachment plaques or desmosomes have been described by several workers using electron microscopy (see Fawcett, 1958, Odland, 1958; Gray 1961*a*). They occur commonly between cells of non-nervous tissues, especially epidermis.

A final feature of the mossy fibre endings is the occasional appearance of bundles of neurofilaments lying in clear cytoplasm (Pl. 3, fig. 3; Pl. 4, fig. 7, *nf.*). In all probability they correspond with the neurofibrillae seen by Cajal (1911) in silver preparations using light microscopy (see Boycott *et al.* 1960, 1961).

In the islands axo-dendritic contacts can also be observed with small presynaptic processes (e.g. *y*, Pl. 3, fig. 3), identified at high magnification by their vesicles. These may be the terminals of the Golgi cells or sections of outlying extensions of the mossy fibre endings. It must also be remembered that a given dendritic process cannot specifically be identified as belonging to a granule cell, for a small proportion of the dendrites entering the islands originate from the cells of Lugaro (see Fox, 1959).

Purkinje cells

Purkinje cells (Text-fig. 1) are not considered in detail here since they have already been studied with the electron microscope by Fernández-Morán (1957) and by Malhotra & Meek (1960). In both these papers the cytoplasm in osmium tetroxide-fixed preparations shows dilatation of the channels of the endoplasmic reticulum with the RNP granules sandwiched between. The author's preparations showed a similar appearance when using the method of preparing small slices before fixation (p. 346). Palay & Palade (1955) warned that Purkinje cells are extremely difficult to fix for electron microscopy. They injected osmium tetroxide into the fourth ventricle and their micrographs of the Purkinje cells show narrow non-dilated endoplasmic reticulum (see also Malhotra & Meek, 1960). It seems that the dilated state is an artefact, which possibly results from the pressure of the blade when the cerebellum is cut up into small slices prior to fixation (see below).

Other features observed by electron microscopy are typical of neurons in general. In osmium preparations the surface membrane appears as the usual dense line from 60–80 Å. thick (see Discussion). The apical dendrite and its finer branches contain the characteristic tubules, about 200 Å. in diameter (Palay, 1956; Gray, 1959*a*, *c*.)

Dendrite spine synapses of the Purkinje cells in the molecular layer

Light microscopy

The molecular layer (Text-fig. 1) consists of vast numbers of unmyelinated axons ascending from the granule cells and equally numerous dendritic branches of the Purkinje cells. These are mixed with a smaller number of the processes of the Golgi

cells, the deep stellate (basket) and superficial stellate neurons and glial processes. In Golgi preparations the more distal Purkinje cell dendrites are covered with thousands of short spines. It is impossible to detect any synaptic contacts between the axons and the various dendritic processes by light microscopy.

Electron microscopy

Electron microscopy reveals numerous synapses in this layer, the majority of which appear to be contacts between axon terminals and dendritic spines. The presynaptic processes (Pl. 7, figs. 17, 19) contain synaptic vesicles, which are invariably aggregated near the thickenings of the synaptic membranes. Mitochondria are occasionally present and a few tubules can sometimes be observed in the preterminal axon where it appears in the plane of section. The post-synaptic processes (Pl. 7, fig. 19, *sp.*) are undoubtedly sections of dendritic spines for they can sometimes be seen as dendritic projections in the plane of section (Pl. 7, fig. 16). Note that in this case one presynaptic process is synapsing with two spines originating from the same dendrite.

The spine tip often lies within an invagination of the presynaptic process (Pl. 7, fig. 17, $sp._1$). When the plane of section is perpendicular to the axis of the spine ($sp._2$, $sp._3$) its section appears completely surrounded by the presynaptic process.

Details of the synaptic cleft of a spine synapse are shown in Pl. 7, fig. 18. The postsynaptic (spine) membrane (*p.m.t.*) appears thicker and denser than the presynaptic membrane and the material (*ma.*) of the synaptic cleft is characteristically situated nearer the post- than the presynaptic membrane. This arrangement is characteristic of type 1 synapses (Gray 1959*c*).

Many of the small processes seen in sections of the molecular layer are not readily identifiable by electron microscopy. Bundles of small profiles (Pl. 7, fig. 19, *u.ax.*), many less than $0{\cdot}5\,\mu$ in diameter, are probably sections of the unmyelinated granule cell axons.

Fixation artefacts in Purkinje and cerebral neurons

Chromophilic neurons in Nissl preparations have long been a puzzle to light microscopists. In a previous section Purkinje cells were described with dilated endoplasmic reticulum when observed by electron microscopy. Picard & Cotte (1959) have shown by light microscopy that pressure on Purkinje cells before fixation can result in chromophilia. In this section the evidence also suggests that pressure before fixation can be responsible for chromophilia in Purkinje and certain cerebral neurons, and that by electron microscopy the affected neurons show dilated endoplasmic reticulum and other features.

In the cerebral cortex certain very dense cells with dilated channels of the endoplasmic reticulum, granules in the compressed cytoplasm, and crenated surface—and nuclear—membranes, can be seen by electron microscopy. These cells were at first mistaken for microglia, but their size and shape (often pyramidal) and the presence of axosomatic synapses left little doubt that they were in fact neurons. They can be distinguished equally well from the paler apparently less shrunken neurons by phase-contrast light microscopy of plastic sections from the same block. The dense neurons occur especially near the cut edge of the tissue slice.

Nissl preparations of similar tissue slices were examined by light microscopy after fixation in 10% neutral formal saline, the brain tissue being cut into slices before fixation just as when fixed with osmium tetroxide for electron microscopy. A zone 100–200 μ wide containing a large proportion of apparently shrunken chromophilic neurons, was observed along the cut surface in sections examined by light microscopy. In further experiments the surface of the cerebral cortex was exposed and pressed upon with the back of a scalpel and the brain was then immediately fixed. Nissl preparations showed a distinct zone of shrunken chromophilic neurons below the pressure line, in some sections extending through the entire depth of the cortical grey matter (about 2 mm. in the rat visual cortex). There seems little doubt that pressure on certain cerebral neurons before fixation can render them chromophilic and shrunken in appearance in Nissl preparations. This artefact can also be observed by electron microscopy. The affected neurons show the dilated channels of the endoplasmic reticulum and other features mentioned above.

All Purkinje cells that have so far been examined by the author using fresh slices for fixation have shown dilated endoplasmic reticulum, etc. When the cerebellar cortex was frozen with carbon dioxide however and then cut into slices in the solid state, thus avoiding any pressure on the Purkinje cells before fixation, the endoplasmic reticulum did not appear dilated. It seems therefore that the Purkinje cells, like certain cerebral neurons, are especially sensitive to the pressure of the razor blade used in preparing the slices.

DISCUSSION

In this study only limited aspects of the cerebellar cortex have been described and much remains to be done. For example, no observations have been made by electron microscopy, on the structure and connexions of the 'Lugaro' neurons (see Fox, 1959) or Golgi cells (Cajal, 1911; Scheibel & Scheibel, 1954) of the granule layer (see Text-fig. 1). Nor have the synaptic contacts on the Purkinje cell surface been examined by electron microscopy. By light microscopy the Purkinje cell body is seen to receive numerous contacts from the basket cell axons (see Cajal, 1911; Scheibel & Scheibel, 1954) and the dendritic trunks to receive contacts from the climbing fibres and the recurrent collaterals of the Purkinje cell axons (see Cajal, 1911; Carrea, Reissig & Mettler, 1947; Szentágothai & Rajkovits, 1959). Finally, there are the stellate and basket cells of the molecular layer, and what may prove of special interest, certain axo-axonal contacts that have been described by light microscopy by Scheibel & Scheibel (1954).

Two especially interesting structures observed in the cerebellar islands are the complex vesicles in the mossy fibre endings and the thickenings on apposed pairs of dendritic membranes. Similar complex vesicles occur commonly in the presynaptic processes of the spinal cord and they can occasionally be observed in such processes of the cerebral cortex. They will obviously need accounting for in any theory concerned with the functioning of presynaptic organelles.

The dendritic thickenings are of two sorts, those at synaptic contacts with axons where the thickenings are asymmetrical and symmetrical thickenings at dendrodendritic contacts. The latter may represent firm attachment regions between the

adjacent dendritic processes, although 'synaptic' interaction between dendrites cannot be entirely excluded. The functions of the asymmetrical thickenings of the synaptic membranes are equally obscure. Since the vesicles aggregate in these regions they are possibly the transmission points. The thickenings in this case are certainly regions of firm attachment since pre- and post-synaptic membranes remain apposed during homogenization and ultracentrifugation, whereas other membrane regions show no adhesion (Gray & Whittaker, 1960). Since these and the dendro-dendritic thickenings can be observed in the same section, histochemical tests (when developed for combination with electron microscopy) might reveal choline esterase only in the former site, for the mossy fibre endings have been shown to have a cholinergic mechanism (Hebb, 1959).

The spacing between processes in sections of the cerebellar cortex is about 200–300 Å. and is similar to that of the cerebral cortex. In both situations extracellular fibrils are absent. It has been suggested that the sodium spaces may not be limited to these narrow extracellular zones, but may also include intracellular regions of glia (see Wykoff & Young, 1956; Schmitt, 1958; Sjöstrand, 1960). This is a useful hypothesis, but there is little evidence to support it at present. Sjöstrand points out that an exploring micro-electrode can move over fairly long distances in what is assumed to be an extracellular region (presumably when no resting potentials are encountered). This cannot be used as evidence to support the above hypothesis, however, for the majority of neuronal and glial processes of the neuropil, at least in the cerebral (Gray, 1959*c*) and cerebellar cortices (see above), are $1\,\mu$ or less in diameter and are thus too small to be entered successfully by the microelectrode tip. They are probably ruptured with loss of polarization, as the electrode approaches.

Another factor to be taken into account is that certain neurons are especially sensitive to pressure and this would explain the difficulties of obtaining intracellular recordings from them. Cajal (1911) described a special thickening of the Purkinje cell membrane revealed by light microscopy in silver chromate preparations. This was thought by Granit & Phillips (1956) to be a possible explanation for the great difficulty in successfully penetrating Purkinje cells with microelectrodes for intracellular recording. Electron microscopy, however, shows only a single 60 Å. dense line at the Purkinje cell boundary thus revealing nothing special about the Purkinje cell membrane. Probably it is the special sensitivity of this cell to the mechanical disturbance of an approaching electrode that leads rapidly to a general disorganization of the cytoplasm with loss of polarization.

Finally, it is clear from the present work that the Purkinje cell spines of the cerebellum are post-synaptic processes just as are the spines of dendrites of the cerebral cortex (Gray, 1959*b*, *c*, 1961*a*). The nature and reality of the spines have for long been debated by light microscopists (see Golgi, 1894; Cajal, 1911; Fox & Barnard, 1957). Fox & Barnard were inclined to the view that the spines were sites of synaptic contact and used their number as an index for a quantitative estimate of the number of synapses on Purkinje dendrites. They were clearly justified in this view, although electron microscopy shows that there is not always a one-to-one relationship between axon and spine.

Unlike the spines of the visual area (Gray, 1959*b*, *c*, 1961*a*) and hippocampal region (Hamlyn, 1961) of the cerebral cortex, the cytoplasm of Purkinje dendritic spines contains no spine apparatus. A third site where dendrite spines are known to

be especially well developed is the caudate nucleus (Fox & Barnard, 1957). This region has not yet been investigated by electron microscopy. The spine apparatus of cerebral neurons occurs near the post-synaptic membrane, but it remains to be shown that it has any connexion with synaptic activity.

SUMMARY

1. The cerebellar cortex of the rat has been fixed with osmium tetroxide, stained with phosphotungstic acid, embedded in Araldite and examined with the electron microscope.

2. The cell bodies and dendrites of the granule neurons are described. No axo-somatic synapses were found.

3. The mossy fibre endings form large bags in synaptic contact with the dendrite tips of the granule and other neurons. Characteristic membrane thickenings occur at the contact regions. The bags contain synaptic vesicles, mitochondria, complex vesicles, and, occasionally, bundles of neurofilaments.

4. Membrane thickenings, distinct from those related to synapses, occur in the cerebellar islands (glomeruli). These are tentatively identified as dendro-dendritic attachment plaques.

5. The spines of the Purkinje cell dendrites are confirmed to be sites of synaptic contact. The spine cytoplasm contains no spine apparatus.

6. Artefacts in Purkinje and other neurons are probably caused by pressure when the tissue is sliced prior to fixation.

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KEY TO LETTERING

a.p. attachment plaque
a.r. agranular reticulum
ax. myelinated axon
c.v. complex vesicle
d.c. dark cell of granule layer
den. dendrite
den.t. dendrite tubule
gr. granules of the endoplasmic reticulum
m. mitochondrion(a)
ma. layer of material in synaptic or attachment-plaque cleft
m.e. membranes of the endoplasmic reticulum
m.t. thickenings of the synaptic membranes
nf. neurofilaments of axon
nuc.g. nucleus of granule cell
sp. dendrite spine
s.v. synaptic vesicles
ton. tonofibrils
u.ax. unmyelinated axon
ves. vesicular profile
x outer regions of granule cell cluster
y small presynaptic process

EXPLANATION OF PLATES

Plate 1

Fig. 1. A group of granule neurons and an island of the granule layer (top right).

Plate 2

Fig. 2. An island (glomerulus) of the granule layer with granule neurons (right and below).

Plate 3

Fig. 3. A mossy fibre presynaptic process seen in continuity with a myelinated axon.
Fig. 4. A mossy fibre ending packed with synaptic vesicles and mitochondria.
Fig. 5. A granule neuron with dendrite.

Plate 4

Fig. 6. Part of the nucleus and cytoplasm of a granule cell.
Fig. 7. Neurofilaments lying in a zone of clear cytoplasm in a mossy fibre ending.

Plate 5

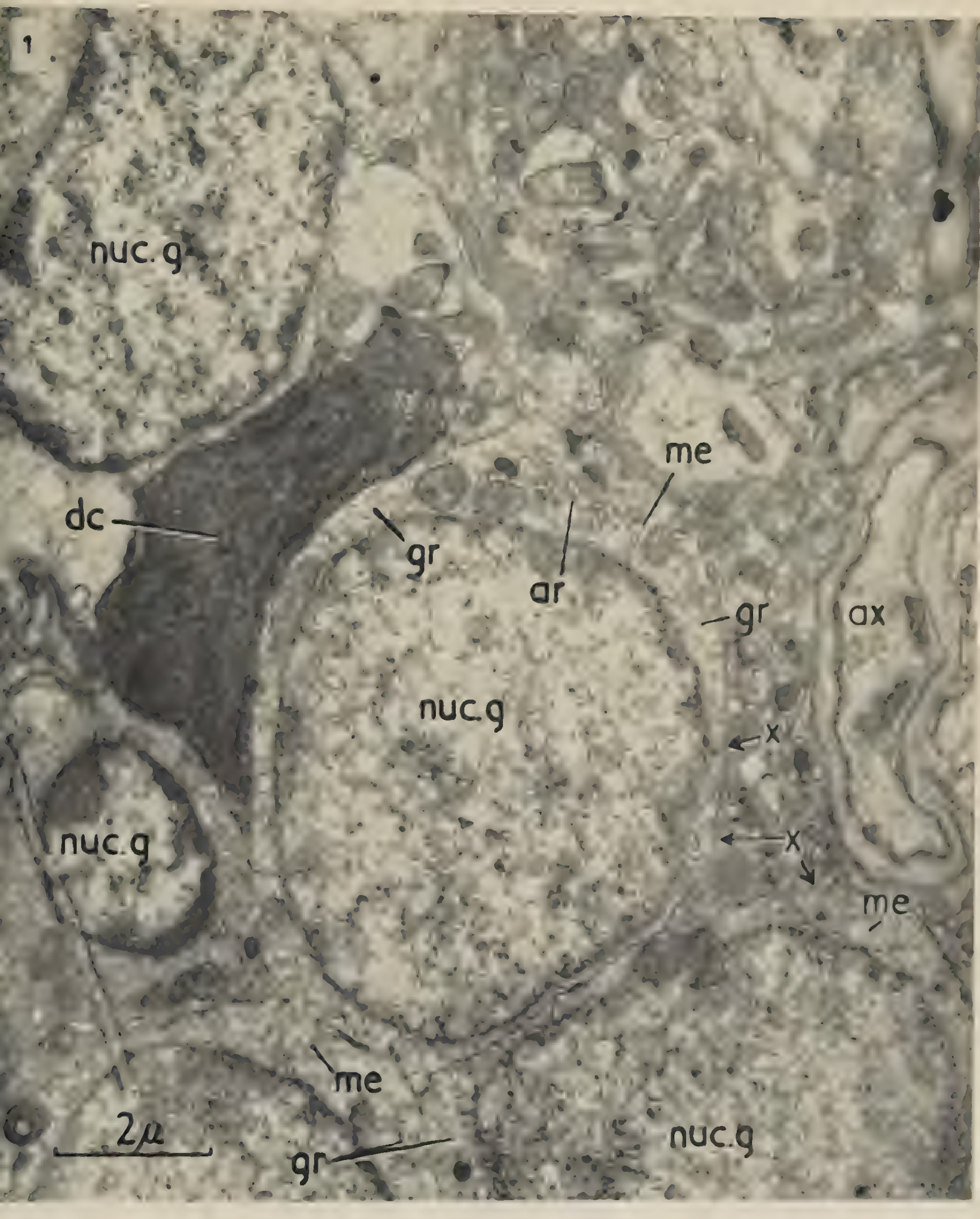
Fig. 8. A mossy fibre ending in the granule layer.

Plate 6

Fig. 9. Thickened region of mossy fibre synapse.
Fig. 10. A dendro-dendritic attachment plaque.
Fig. 11. A dendro-dendritic attachment plaque.
Fig. 12. Complex vesicles of a mossy fibre ending.
Fig. 13. Complex vesicles of a mossy fibre ending.
Fig. 14. Thickened region of mossy fibre synapse.
Fig. 15. A peculiar body containing synaptic vesicles and complex vesicles found within a mossy fibre ending.

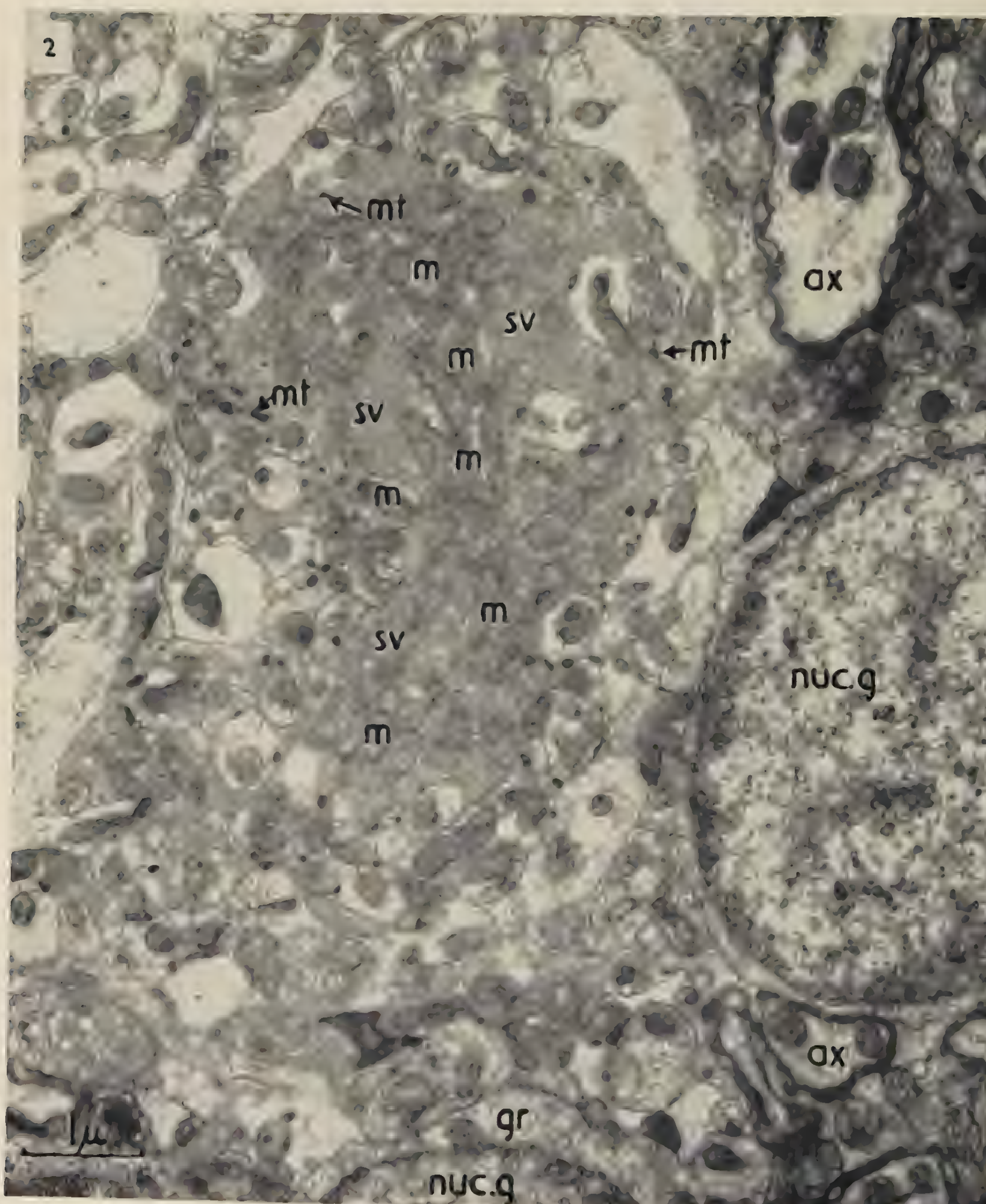
Plate 7

Fig. 16. Purkinje dendrite with two spines. They are in contact with a single presynaptic process.
Fig. 17. Spine synapses of Purkinje cells. The synaptic processes are cut in various planes.
Fig. 18. The synaptic membranes of a Purkinje spine synapse.
Fig. 19. Sections through the tips of a group of four Purkinje dendrite spines. Their presynaptic processes contain the characteristic synaptic vesicles.



GRAY—Fine structure of cerebellum

(*Facin*, p. 356)



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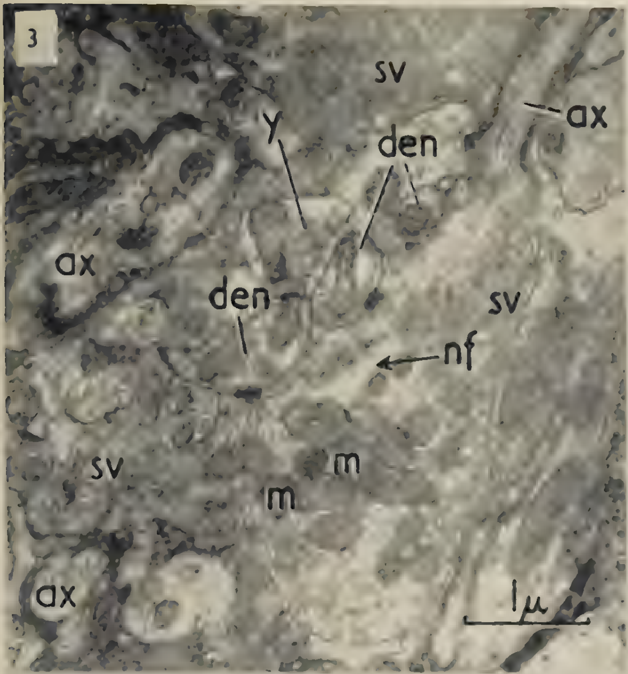

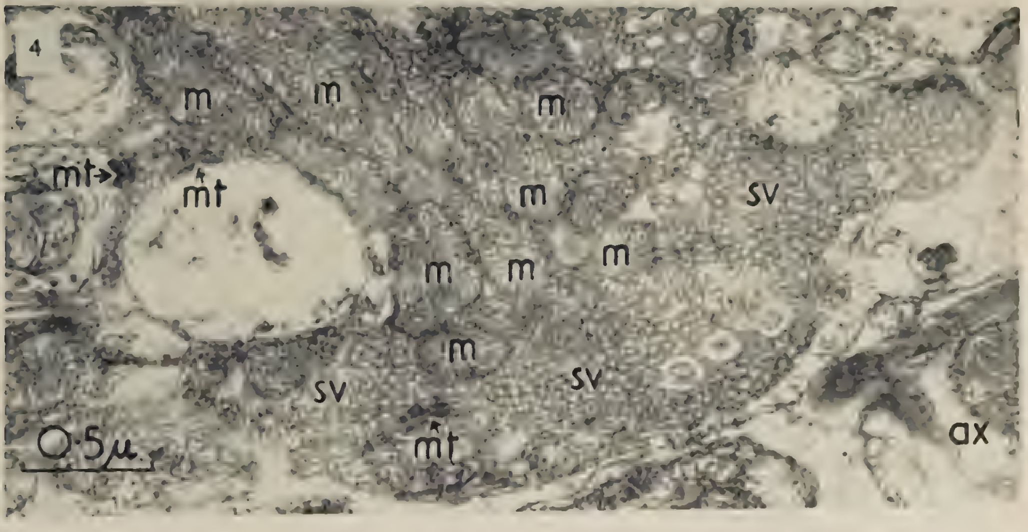

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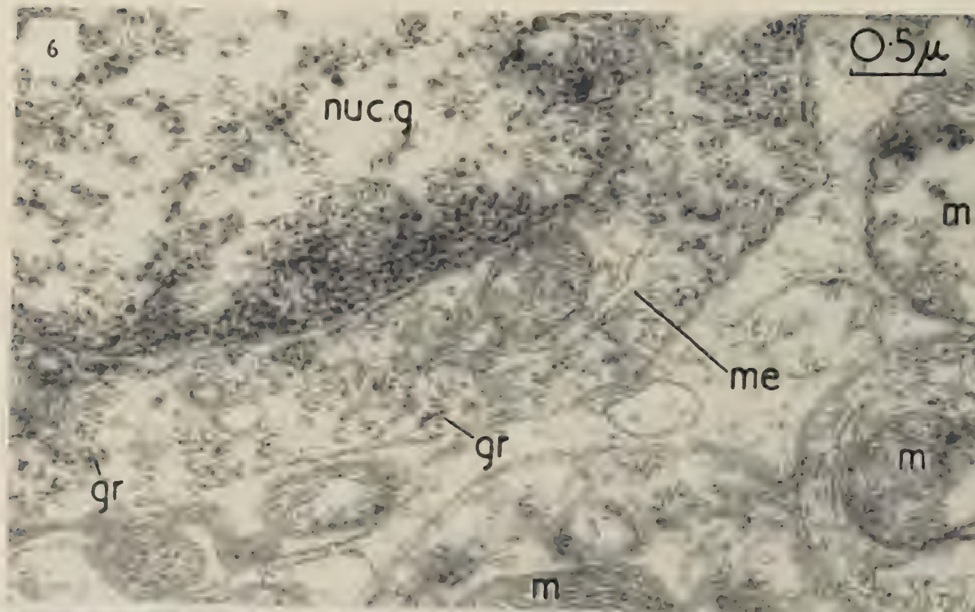
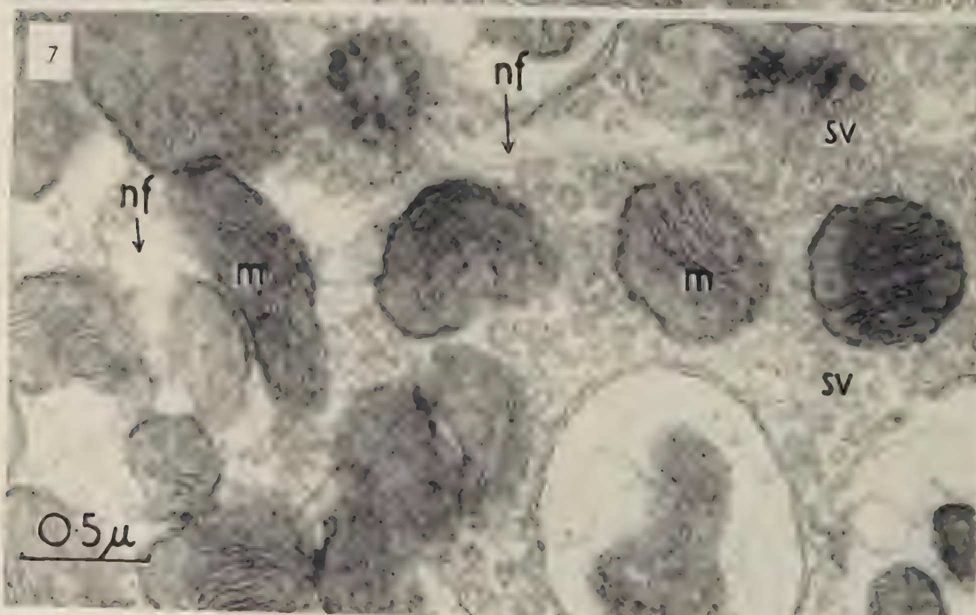
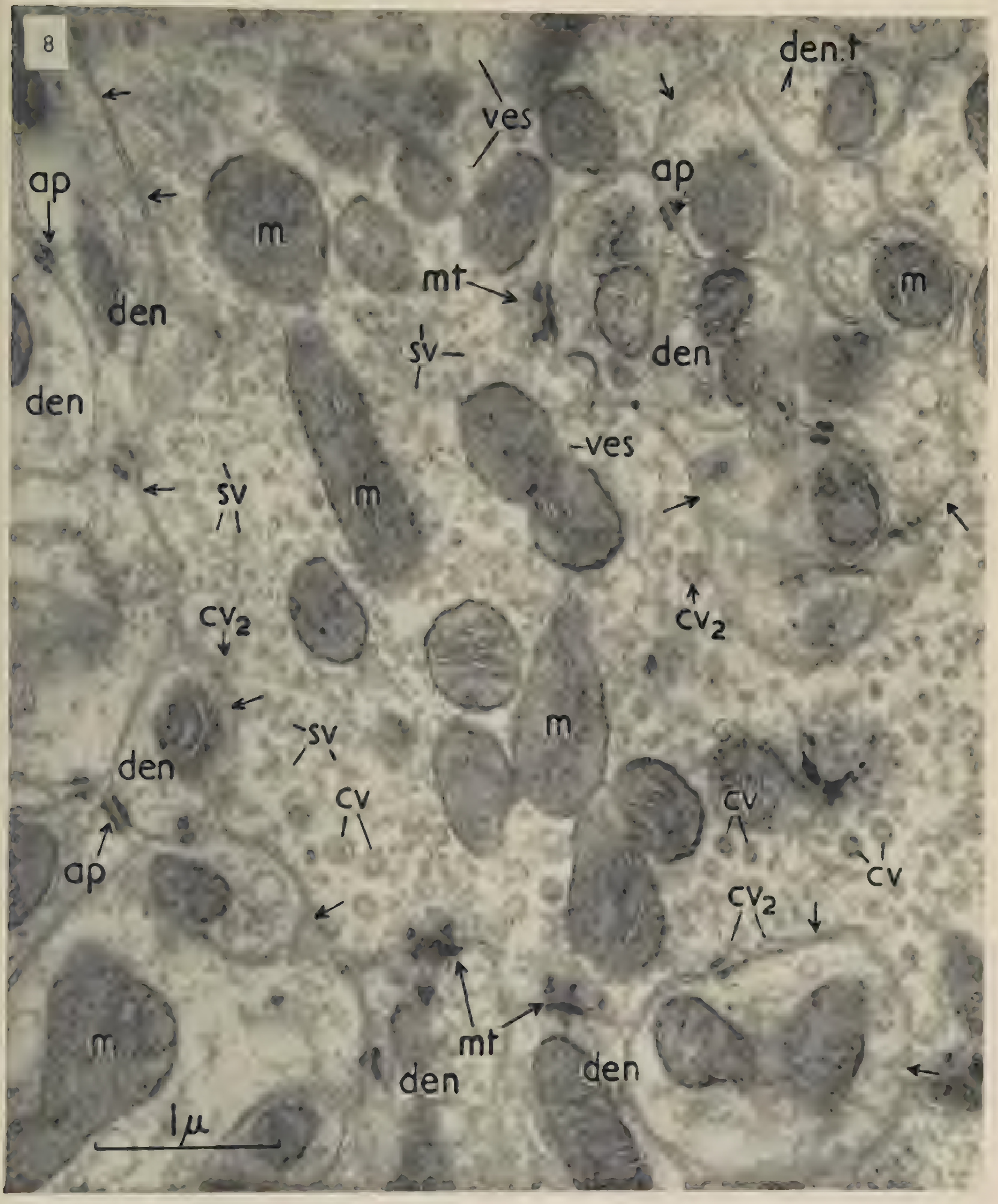




GRAY—Fine structure of cerebellum



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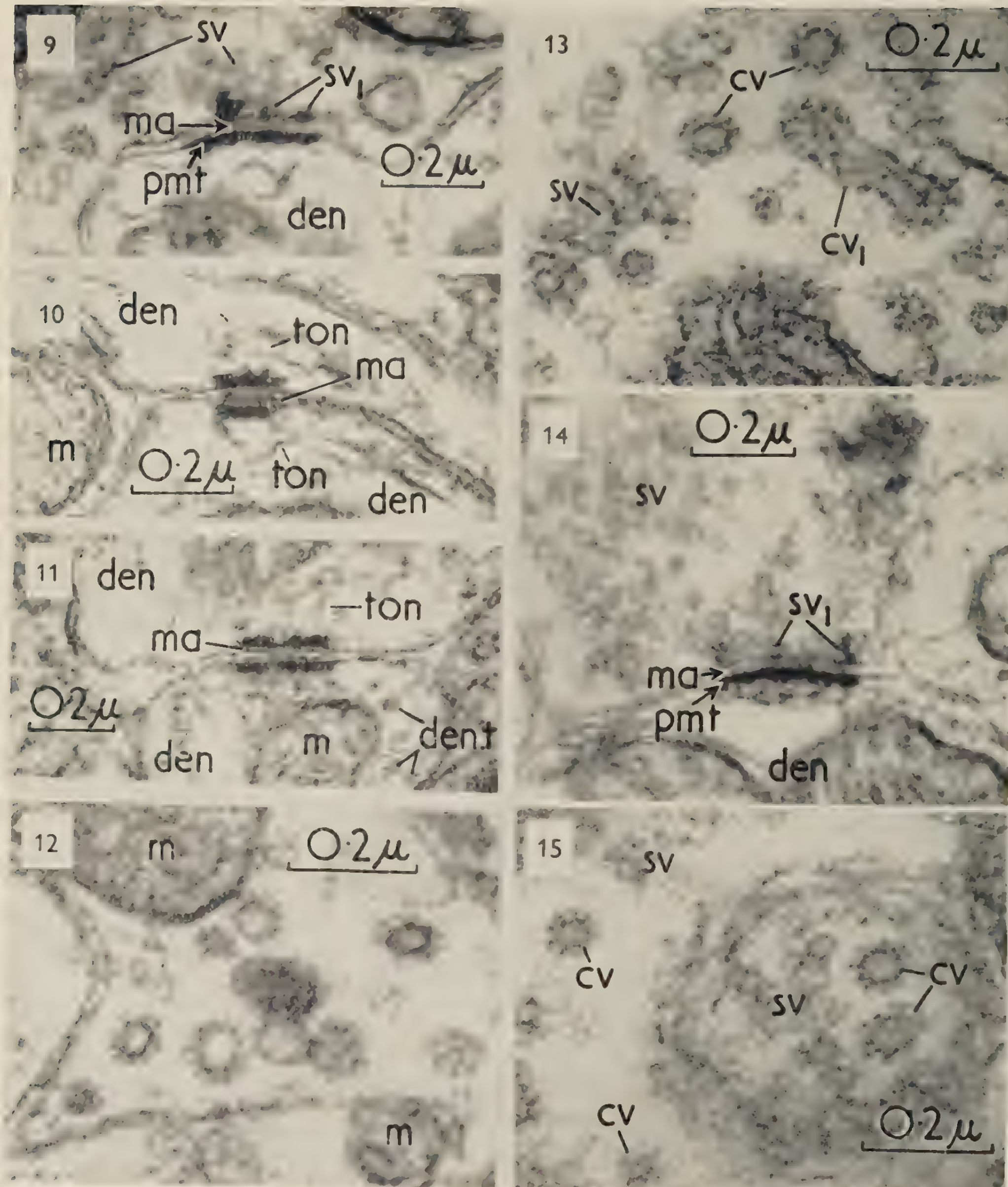
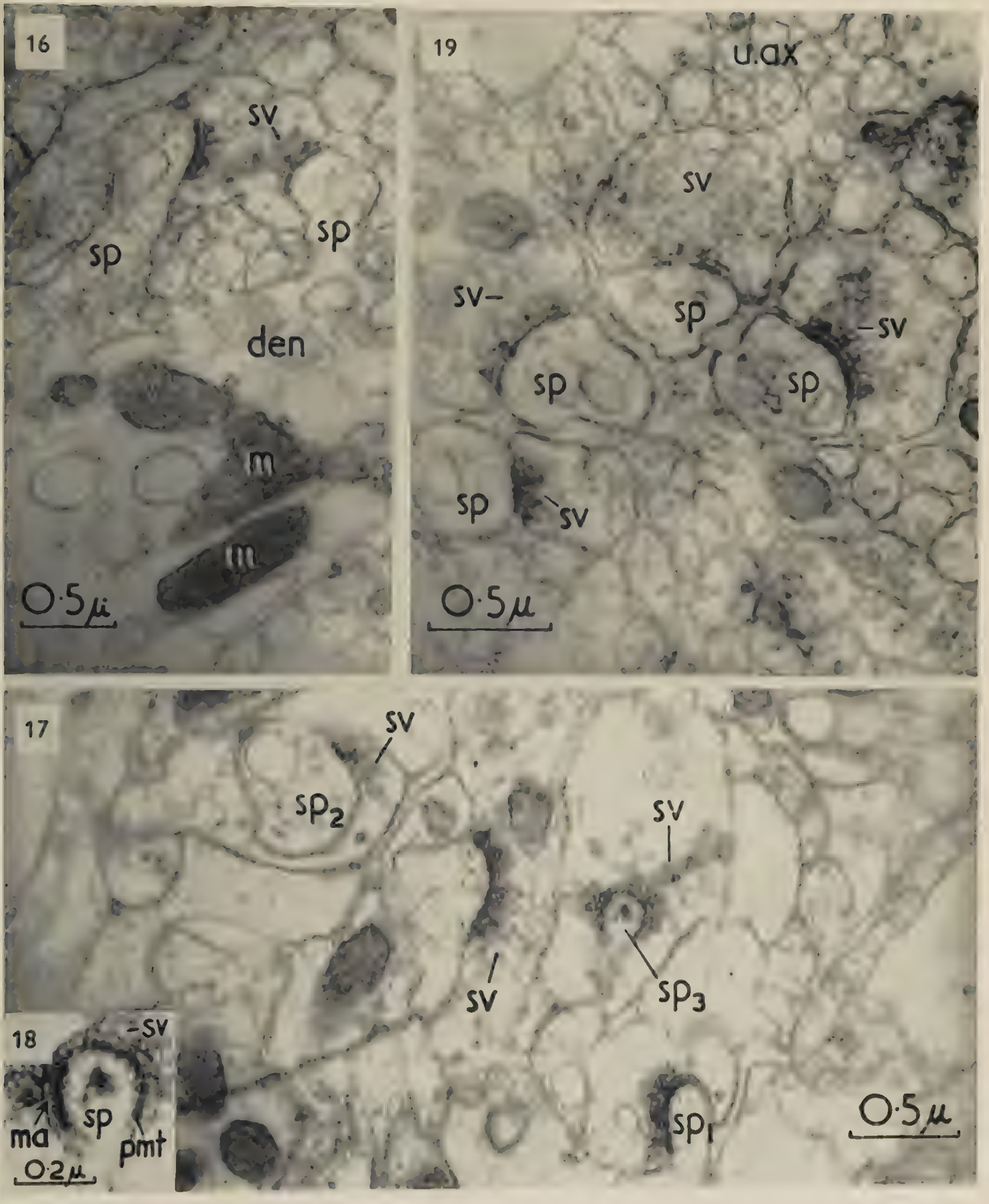


GRAY—Fine structure of cerebellum



GRAY—FINE STRUCTURE OF CEREBELLUM

OBSERVATIONS ON THE GROWTH AND HISTOCHEMISTRY OF THE LEYDIG TISSUE IN THE POSTNATAL PREPUBERTAL MOUSE TESTIS

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Two reasonably distinct generations of Leydig cells have been described in most mammalian species so far studied: the first is a foetal generation, while the second is usually described as arising around puberty. Following Gillman (1948) it is now widely accepted that the foetal Leydig cell arises from a mesenchymal cell. During cytomorphosis the oval nucleus of this precursor becomes rounded and vesicular, while the chromatin becomes localized just inside the nuclear membrane. Thereafter the cytoplasm increases in amount, its processes diminish in size and number and the cell body assumes a polygonal epithelioid appearance. Lipid droplets appear at a variable point during this process.

In man and horse (Gillman, 1948) foetal Leydig cells are supposed to reach a developmental maximum at or about mid-term; thereafter the Leydig tissue undergoes a regression, the occurrence and timing of which has not been satisfactorily explained. By contrast in the rat (Roosen-Runge & Anderson, 1959), rabbit (Allen, 1904) and pig (Bascom & Osterud, 1927) similar Leydig tissue maxima do not occur until birth. The ensuing regression in these animals has been ascribed to deprivation of maternal oestrogens and gonadotrophins. The Leydig cell of the foetal mouse has not been investigated in this connexion.

The intertubular tissue of the postnatal, prepubertal testis in most mammalian species, for example the bull (Hooker, 1944) is said to show little evidence of differentiation and consists mainly of mesenchymal cells. Many of these cells, however, contain typical Leydig nuclei (Hooker, 1948). While it is widely accepted that the second, or postnatal, generation of Leydig cells makes its first appearance at puberty or at least undergoes a tremendous increase in cell number about that time, Hooker has pointed out that this generalization is based on surprisingly few observations.

Close scrutiny of the literature on the life history of the Leydig cell indicates that our knowledge is, at the best, fragmentary and many aspects remain to be explored. The present investigation was undertaken in an attempt to ascertain whether there are two entirely separate generations of Leydig cells, foetal and pubertal, and if so, whether the pubertal generation in fact arises as the result of massive cell division or cell differentiation. Furthermore, it was hoped to establish whether or not a fully differentiated Leydig cell can undergo mitosis. Finally the relationship of histochemical changes in the Leydig cell to its androgenic function during the prepubertal period requires clarification.

MATERIAL AND METHODS

The work may conveniently be divided into two parts: (1) a description of the histology and histochemistry of the pre-pubertal testes and seminal vesicles; (2) observations of colchicine arrested mitoses in prepubertal and adult testes.

The animals used were albino mice aged from 0 days to 8 weeks; thirty three mice were sacrificed in the preparation of the age series, twenty-two were used in the colchicine study. Puberty, in the mouse, occurs about the fourth week of extra-uterine life but differentiation of the secondary sexual organs is not complete, however, until the eighth week of postnatal life. For this reason cytological and cytochemical studies were extended to include animals up to 8 weeks old notwithstanding the fact that the testis reaches its adult size by the end of the sixth week of extra-uterine life.

In preparation of the age series the mice were killed at 0, 7, 14, 21, 28, 35, 42, 49 and 56 days. Two testes from each age group were subjected to each of the following procedures:

(1) A modified McManus/Hotchkiss periodic acid-Schiff (PAS) reaction (Baillie, 1960*a*) which minimizes testicular shrinkage and distortion of Leydig tissue.

(2) Haematoxylin and eosin staining of sections prepared for the P.A.S. procedure.

(3) Coloration with Sudan black to demonstrate total lipids present after formol calcium fixation.

(4) Haye's modification of Feulgen and Voit's plasmal reaction (Lillie, 1954*a*) to show acetal phosphatides and, possibly, steroids (Dempsey, 1948).

(5) Staining with 2:4-dinitrophenyl hydrazine (Albert & Leblond, 1946).

(6) Fixation in 70% cold ethanol for demonstration of alkaline phosphatase (Lillie, 1954*b*).

One testis from each of the first six arbitrary age groups was also stained for mitochondria after fixation in Helly's fluid.

Testicular volume was measured on gonads fixed in formol calcium using the formula $V = 4/3\pi b^2 a$, where $V =$ testicular volume, $b =$ half the equatorial diameter of the testis and $a =$ half its polar diameter (Harrison & MacMillan, 1954). The measurements were carried out under a microscope using a scale calibrated in $\frac{1}{100}$ mm. In common with the observations of Harrison & MacMillan (1954) it was found that measurements on the unfixed testis were inaccurate since the gonad flattens when placed on a hard surface: agitation during the initial stages of fixation obviates this source of error. Preliminary work indicated that there was gross Leydig tissue shrinkage and distortion after alcoholic fixation (cf. Pl. 1, fig. 2 and Pl. 3, fig. 9) and, moreover, relatively large testes (e.g. those of the adult hedgehog and rat) were frequently irregularly fixed in their central portions. Formalin fixed mouse testes were largely free of these artefacts.

At the end of the fourth week of extra-uterine life, when the seminal vesicles become large enough for weighing, they were dissected out, their outlets clamped and the entire preparation fixed in formol corrosive solution for 6 hr. and then weighed. Since fluctuations in androgen release are thought to be reflected by altered secretory activity in the seminal vesicles (Baker, Schairer, Ingle & Li 1950), it was considered advisable to preserve all of the secretion for weighing. On this basis it was felt that

attempts to weigh the fresh vesicle were unsatisfactory due to partial loss of secretion owing to agonal contraction, and that the weight of the fixed vesicles provided a truer figure for comparison. Vesicles were routinely stained for alkaline phosphatase and with haematoxylin and eosin.

All mature Leydig cells were found to have sudanophilic cytoplasm. Numerous random sections of testes from each age group coloured with Sudan black were projected by microscope on to paper, when the areas of black tissue (i.e. Leydig cells) were marked out by pen. The relative volume of Leydig tissue was derived as a percentage by weighing the total paper field and, later, the cut-out areas representing Leydig tissue. The total volume of Leydig tissue was calculated from the percentage and the testicular volume. In a similar fashion the volume of Leydig tissue containing plasmalogens (Schiff stainable lipids; acetal phosphatides and possibly steroids) was also calculated. The relative and total volumes of intertubular tissue were estimated on P.A.S. stained material.

Twenty-two animals were used in the colchicine study, being sacrificed in pairs between birth and the end of the eighth week of postnatal life: in addition, four adult animals were surveyed. Each animal received an intra-peritoneal injection of colchicine (0·1 mg. in water/100 g. body weight) at 10.00 a.m. on the appropriate day. They were killed 5 hr. later and their testes excised for examination using the PAS technique. The period was identical with that used by Ebling (1954) and Bullough (1950) who selected it to vitiate the effects of the diurnal mitotic cycle. The mitotic rate was calculated by counting the total number of typical Leydig cells in a given field and also the number of Leydig cells in the same field whose nuclei were in arrested mitosis: from these figures the mitotic rate is derived as a percentage per 24 hr.

RESULTS

(1) *Testicular volume*

As will be seen from Text-fig. 1, the testicular growth curve is sigmoid in form. Growth is completed by the time the animal is 6 weeks old.

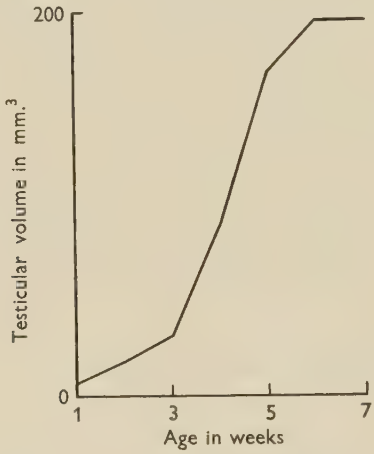
(2) *Haematoxylin and eosin observations*

At birth the intertubular tissues account for over 70% of the testis. Typical, stellate or spindle-shaped mesenchymal cells, exhibiting occasional mitotic figures, are linked by transitional forms with peritubular fibroblasts on the one hand and Leydig cells on the other. The Leydig cells usually occur in groups or clumps. Their cytoplasm is abundant, rounded or hexagonal in shape, strongly eosinophilic (Pl. 1, fig. 1), and is not vacuolated. The cell membrane is clearly defined. The eccentric nucleus is round or ovoid and its membrane is lined with chromatin which also forms four or five heavily staining granules in the interior.

At the end of the first week, the intertubular tissue is seen to be apparently much reduced, the Leydig cells are less readily visible, and their cytoplasm has become vacuolated. This apparent atrophy is still evident at the end of the second week, but during the third, fourth and fifth weeks the Leydig cells become much more prominent. Their vacuolated foamy cytoplasm contains small numbers of minute yellow refractile granules which appear to increase in number as the animal approaches maturity. There is no significant change during the sixth, seventh and eighth weeks.

(3) *Periodic acid-Schiff reaction*

The neonatal Leydig cell cytoplasm does not stain with the PAS reaction. A few cells have PAS positive material either lining part of the cell membrane or forming four or five juxta nuclear granules. The staining propensity of this material is unaffected by treatment with diastase or with the lipid solvents. A few peculiar cells with pycnotic nuclei and scanty cytoplasm, which is strongly PAS positive, are to be seen. There is no demonstrable PAS positive, intercellular ground substance.



Text-fig. 1. The testicular growth curve is sigmoid in form and growth is complete by the end of the sixth week of postnatal life.

In testes taken from older animals the individual Leydig cells show a constant picture: their foamy cytoplasm is very faintly PAS positive in sharp contrast with the strongly PAS positive ground substance. Quite a few cells contain numerous bright red, diastase-resistant, refractile granules. The shrunken cells with pycnotic nuclei and PAS positive cytoplasm are fairly common in the 7-day-old testis, but they are rarely visible in older gonads. As the mice mature the connective tissue ground substance becomes clearly defined by the PAS procedure and contains PAS positive material which resists digestion with amylase and extraction with chloroform (Pl. 1, fig. 2). The individual Leydig cells are separated by ground substance which in places contains large oval or round spaces.

(4) *Sudan black observations*

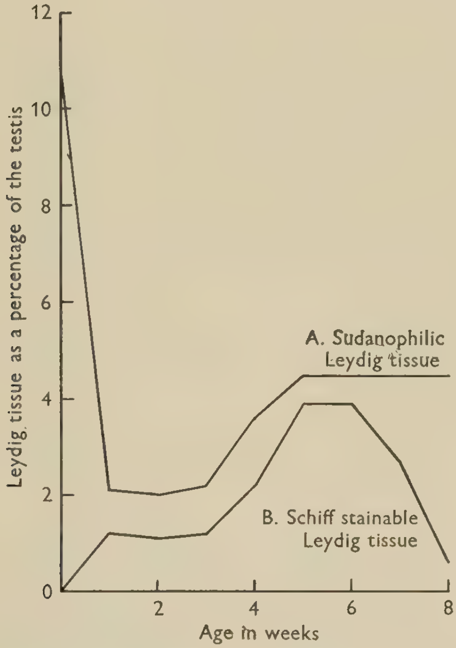
At birth (Pl. 1, fig. 3) the mouse testis interstitium contains abundant quantities of sudanophilic material, which, on closer insection, is seen to consist of numerous minute lipid droplets dispersed through the Leydig cytoplasm. The cell nucleus is rarely obscured by the masses of lipid droplets and the cell boundaries are prominently defined. Undifferentiated mesenchymal cells have no visible lipids: transitional forms are common. The testes of 7-, 14- and 21-day-old mice present a uniform picture (Pl. 1, fig. 4). The amount of interstitial sudanophilic material has under-

gone a marked apparent reduction (c.f. Text-fig. 2), and the individual cells are rounded or polygonal. Transitional forms, are as before, still visible.

The interstitium of later testes (from mice aged 4, 5, 6, 7 and 8 weeks) is similar (Pl. 2, fig. 5): the lipid laden Leydig cells have undergone a marked relative increase and are strikingly prominent.

(5) *The plasmal and phenyl hydrazine reactions*

The neonatal Leydig cells contain no Schiff stainable lipids in contrast to all subsequent age groups. Sections of 7-, 14- and 21-day-old gonads are alike (Pl. 2, fig. 6) when exposed to Schiff's reagent. Many groups of Leydig cells stain an intense



Text-fig. 2. The sudanophilic Leydig tissue, when portrayed as a percentage of the testis, is apparently reduced in volume during the first week of extra-uterine life and remains at a low level for 3 weeks before undergoing an apparent increase to reach the adult level. Neonatal Leydig tissue has no Schiff stainable lipids. Thereafter the relative volume of Schiff positive Leydig tissue behaves in the same fashion as that of the sudanophilic Leydig tissue until the seventh week when Leydig cell plasmalogens are strikingly reduced.

purple colour and their cytoplasm contains swarms of minute purple-coloured droplets which tend to obscure details of the cell nucleus and boundaries. During the fourth and fifth weeks of postnatal life the number of cells in the interstitium with Schiff stainable lipids undergoes a remarkable increase which is maximal at the end of the sixth week (Text-fig. 2; Pl. 2, fig. 7); Leydig cell plasmalogens are then remarkably reduced in the ensuing 2 weeks, reaching the characteristic low adult level (Pl. 2, fig. 8) by the end of the eighth week. While this striking reduction in Leydig cell plasmalogen is occurring, abundant Schiff stainable lipids make their

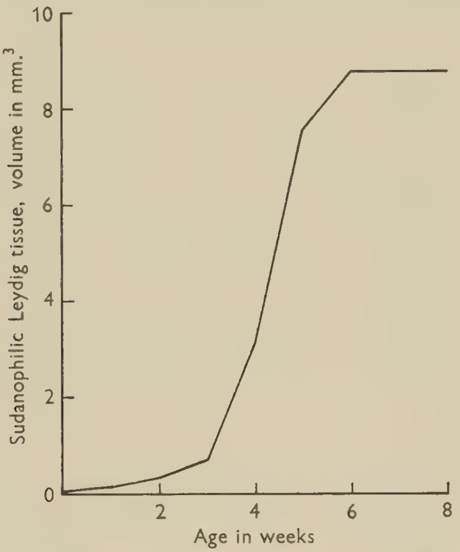
Table 1. *Mensural data of testes and seminal vesicles together with an analysis of intertubular and Leydig tissues*

Age in days	0	7	14	21	28	35	42	49	56
Testicular volume (average) in mm.^{3}	0·8	6·1	18·3	31·7	88·9	169·2	195·4	196·1	196·1
Testicular volume (range) in mm.^{3}	0·6–1·0	5·7–6·4	17·6–19·0	31·0–32·4	88·1–90·0	168·1–170·0	194 4–196·0	195 5–197·0	195·5–196·8
Polar diameter (average) in mm.	1·68	3·02	4·4	5·25	7·68	9·9	9·9	9·9	9·9
Equatorial diameter (average) in mm.	0·95	1·97	2·82	3·4	4·7	5·86	6·14	6·15	6·15
Seminal vesicle weight (average) in mg.	—	—	—	—	12	25	112	218	230
Seminal vesicle weight (range) in mg.	—	—	—	—	10–13	21–28	107–116	204–230	220–245
Logarithm of seminal vesicle weight	—	—	—	—	1·0792	1·3979	2·0492	2·3385	2·3617
Intertubular tissue, %	71·3	17·0	6·1	4·1	5·3	5·5	5·3	5·4	5·4
Sudanophilic Leydig tissue, %	10·6	2·1	2·0	2·2	3·6	4·5	4·5	4·5	4·5
Sudanophilic Leydig tissue, volume in mm.^{3}	0·08	0·13	0·37	0·7	3·2	7·6	8·8	8·8	8·8
Logarithm of sud. Leydig tissue volume	$\bar{2}$·9031	$\bar{1}$·1139	$\bar{1}$·5682	$\bar{1}$·8451	0·5051	0·8808	0·9445	0·9445	0·9445
Schiff stainable Leydig tissue, %	0	1·2	1·1	1·2	2·2	3·9	3·9	2·7	0·6
Schiff stainable Leydig tissue, volume in mm.^{3}	0	0·07	0·20	0·38	1·96	6·59	7·6	5·28	1·18
Leydig mitotic rate/24 hr., %	6·7	6·2	5·3	6·7	6·7	6·2	1·1	0·1	0·0

appearance in the Sertoli cells of the seminiferous tubules. This lipid (and lipid it must be since it dissolves in the fat solvents) does not colour with Sudan black. 2:4-Dinitrophenyl hydrazine gives the same results as Schiff's reagent in the testis.

(6) *Quantitative histological observations*

The quantitative data are summarized in Table 1. The fluctuations in the relative volumes of sudanophilic Leydig tissue and of Schiff positive Leydig tissue are shown in Text-fig. 2. From the testicular volume at the appropriate age (Table 1) the relative volumes of sudanophilic and of Schiff positive Leydig tissue can be translated into absolute volumes (Text-fig. 3 and Table 1).



Text-fig. 3. In contrast to the relative volume (Text-fig. 2), the absolute volume of sudanophilic Leydig tissue undergoes a continuous regular increase between birth and adult life. The growth curve is sigmoid in form.

From Text-fig. 2 we see that the sudanophilic Leydig tissue undergoes a marked apparent reduction in volume during the first week of extra-uterine life. It remains at this low level for 3 weeks before undergoing an apparent increase to reach the adult level. No Leydig tissue contains Schiff stainable lipids in the neonatal testis. Thereafter the relative volume of Schiff positive Leydig tissue behaves in much the same fashion as that of the sudanophilic Leydig tissue, although at any given time the volume of Schiff positive Leydig tissue is appreciably less than that of sudanophilic Leydig tissue. During the seventh and eighth weeks of postnatal life Leydig cell plasmalogens are strikingly reduced.

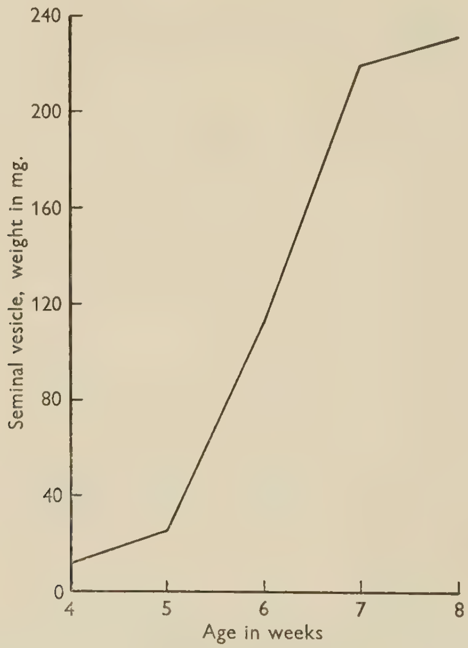
Reference to Text-fig. 3 indicates that while fluctuations take place in the relative Leydig tissue volume, the absolute volume shows a continuous regular increase. The growth curve is sigmoid in form.

(7) *Alkaline phosphatase observations*

The neonatal testis contains no demonstrable alkaline phosphatase; this enzyme makes its appearance in the fibroblast sheath which surrounds the basement membrane of the seminiferous tubules at the end of the third week. It is thereafter a prominent characteristic of the basement membranes of the blood vessels and of the seminiferous tubules. The Leydig cells never contain alkaline phosphatase (Pl. 3, fig. 9).

(8) *Mitochondria*

Throughout the age series mitochondria are visible as minute round refractile granules which fill variable areas of cytoplasm: the perinuclear area is devoid of mitochondria. Many cells are vacuolated, mitochondria being absent from the vacuolated region.



Text-fig. 4. The seminal vesicle weight increases rapidly between the fourth and seventh weeks of postnatal life and the growth curve is sigmoid in form.

(9) *Seminal vesicle weight*

The salient features are summarized in Table 1 and in the sigmoid growth curve portrayed in Text-fig. 4. The vesicles are not large enough for weighing until the animal is 4 weeks old; growth thereafter is largely completed by the end of the seventh week of extra-uterine life.

(10) *Seminal vesicle cytology*

During the initial period of growth, between the fourth and sixth weeks of postnatal life, the principal changes which take place are proliferation of the connective tissue stroma, an increase in the height of the columnar epithelium lining the organ, and the appearance of alkaline phosphatase in the stroma and in the basement membrane of the epithelium (Pl. 3, fig. 11). It seems likely that the later increases in seminal vesicle weight, between the sixth and eighth weeks, are mainly due to the storage of an eosinophilic secretion in the lumen and medial ramifications, or diverticula, of the vesicle. Failure to fix the gland before weighing results in the vesicle presenting a collapsed shrunken appearance suggestive of little secretory activity on account of the extrusion of secretion.

(11) *Results of colchicine administration*

It was found that mice seven days old tolerated the stated dose poorly; in all, some five animals of this age were sacrificed before two were found which survived a five hour period. All other age groups seemed unperturbed by the administration of colchicine. Mitotic figures are readily seen in typical Leydig cells following colchicine administration. To facilitate comparison with the daily increment in Leydig tissue volume, the calculated Leydig mitotic rate is expressed as a percentage (Table 1).

The daily mitotic average during the first 5 weeks of postnatal life works out at 6·3 %. Thereafter it falls progressively: no mitotic figures were seen in adult testes. An example of Leydig mitotic activity is to be seen in Pl. 3, figs. 12 and 13.

DISCUSSION

The mouse Leydig cell is morphologically typical of the mature mammalian Leydig cell having abundant eosinophilic cytoplasm and a rounded vesicular nucleus. The minute granular mitochondria correspond in shape and size with those described by Rasmussen (1932) in the Leydig cell of the cat, mouse and guinea-pig. They are not, however, restricted to the peripheral cytoplasm as suggested by Fawcett & Burgos (1956). The shape, size and distribution of mitochondria is unaffected by age or alterations in lipid distribution. The cytomorphosis of Leydig cells from mesenchymal cells involves the progressive acquisition of mitochondria.

When studied using routine H. & E. staining the Leydig cells are prominent at birth, difficult to discern during the ensuing 2 weeks and become prominent again at the onset of sexual maturity thereby supporting Hooker's (1948) statement that: 'In most species the intertubular tissue of the postnatal, prepubertal testis, shows little differentiation.' The use of the PAS technique however, renders Leydig cells not only visible, but prominent during the entire postnatal prepubertal period, indicating continuity of the supposed foetal and pubertal generations. Moreover, reference to Text-figs. 2 and 3 indicates that, while relative fluctuations in the Leydig tissue volume take place, throughout the postnatal prepubertal period, the absolute volume shows a continuous regular increase. From these facts one must conclude that there is only one generation of Leydig cells in the postnatal mouse and that

the concept of separate foetal and pubertal generations of Leydig cells is based on inadequate histological methods for demonstrating the relatively slowly growing Leydig tissue in the rapidly expanding prepubertal testis.

When stained with the PAS procedure, mouse Leydig cytoplasm stains a pale pink colour and may contain a number of minute red refractile glycoprotein granules. This corresponds with the view (Baillie, 1958) that homoiothermal vertebrate Leydig cells contain carbohydrate-protein complexes acquired after birth, but no glycogen. The Leydig cells of poikilotherms, on the other hand, contain visible glycogen granules. Peculiar shrunken crenated cells with pycnotic nuclei and intensely PAS positive cytoplasm are to be seen in the intertubular spaces of the mouse testis. They are similar to the atrophic cells described in the intertubular tissue of the foetal sheep testis (Baillie, 1960*b*).

In common with the Ledyig cells of the sparrow, chaffinch, greenfinch and Leghorn cockerel (Lofts & Marshall, 1956), deer (Wislocki, 1949) and man (Montagna & Hamilton, 1952; Mancini, Nolazco & Balze, 1952), the mouse Leydig cells contain abundant droplets of sudanophilic lipid. This contrasts with some species of rat (Lofts & Marshall, 1956; Lynch & Scott, 1951) which possess no sudanophilic material in their intertubular tissue. During differentiation from its mesenchymal precursor the Leydig cell acquires minute droplets of lipid which coalesce progressively giving rise to the large granular masses characteristic of mature Leydig cells. At all times in the prepubertal testes fully differentiated Leydig cells with sudanophilic cytoplasmic inclusions exist, further evidence against the widely held concept of separate foetal and pubertal Leydig cell generations discussed above. There is no qualitative change in the sudanophilic interstitial elements which can be correlated with the onset of sexual maturity.

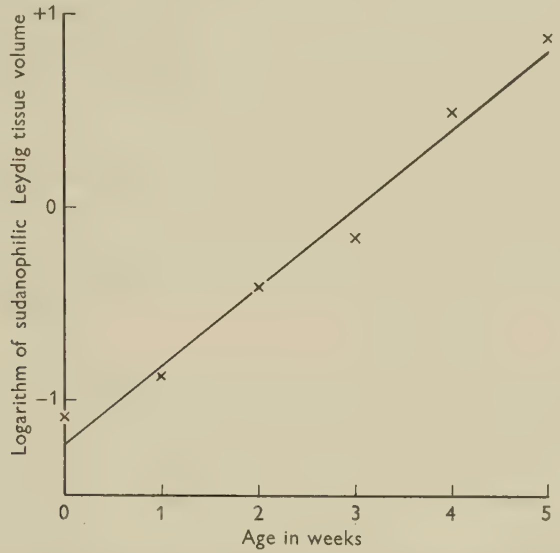
The observations of Albert & Leblond (1946) on the reactivity of the mouse Leydig tissue to Schiff's reagent and 2:4-dinitrophenyl hydrazine have been confirmed with two reservations; first, the neonatal testis contains no complex lipids stainable with these reagents and secondly the mature animal's Leydig cells contain much less of these materials than do the immature animal's cells (Pl. 2, figs. 6–8). The distribution of plasmalogens appears to depend on age and species factors and is also influenced by environmental conditions (Baillie, 1961).

In contrast to human Leydig cells (Mancini *et al.* 1952) and to the Leydig cell of rutting deer (Wislocki, 1949) the mouse Leydig cell is devoid of alkaline phosphatase at all stages of its development.

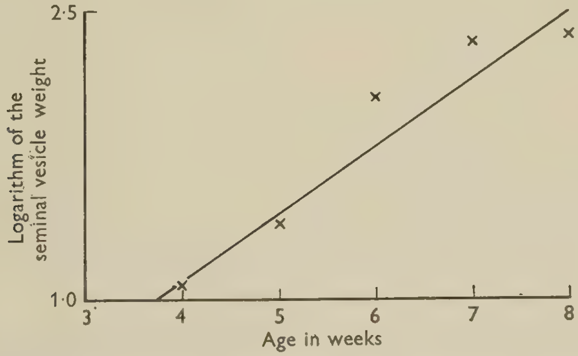
It is generally accepted (Burrows, 1949) that seminal vesicle weight alterations reflect accurately fluctuations in androgen release by the testis, despite Hooker's (1942) suggestion that tissue sensitivity to circulating androgen is raised at puberty. Seminal vesicle growth appears to be unrelated to any morphological or histochemical events occurring in the seminiferous tubules; the appearance of Sertoli cell plasmalogens coincides approximately with the end of seminal vesicle growth, and is thus unlikely to bear any relation to androgen synthesis.

On the other hand, from Text-fig. 3 it will be seen that the total volume of Leydig tissue undergoes a regular increase during extra-uterine life. This increase is completed by the end of the sixth week and is seen to antedate, if compared with Text-fig. 4, a comparable seminal vesicle growth pattern. From the similarity of these

two graphs, together with their positions relative to the time axis, it would seem that the growth of the androgen dependent tissue might be related to the growth in volume of Leydig tissue.



Text-fig. 5. The sudanophilic Leydig tissue volume grows at a rate of 0·0587 log units per day (14·5 %) between birth and the end of the fifth week: the standard error of the straight line is ±0·004 log units per day (±1 %).



Text-fig. 6. The seminal vesicle weight increases at a rate of 0·05 log units per day (12·2 %) between the fourth and eighth weeks of postnatal life: the straight line carries a standard error of ±0·087 log units (±2 %) per day.

This impression is strengthened when one compares the corresponding log graphs (Text-figs. 5 and 6), which are corrected for standard error, and finds that the gradients, which are indices for the growth rates of the two tissues, are similar.

It is notable that the striking reduction in Schiff stainable lipids during the seventh and eighth weeks of postnatal life has no effect on the regularity of seminal vesicle growth. Since Schiff's reagent stains acetal phosphatides and, theoretically

at least, steroids but not neutral fats, it is probable that Leydig plasmalogens are in some way related to androgen metabolism. Androgen production includes synthesis from cholesterol or acetate (Heard *et al.* 1956) storage, and release into the blood stream. The varying levels of Schiff stainable lipids in the Leydig tissue may reflect variations in the amount of hormone or its precursors being stored at different periods. Similar phenomena have been described in the adrenal of the hibernating ground squirrel (Zimny, 1959) and also in the testes of cold stressed adult mice (Baillie, 1961).

Mitotic figures in the postnatal Leydig cell have only been described in inflammatory reactions and in tissue culture experiments (Rasmussen, 1932) though they are numerous in foetal material (Roosen-Runge & Anderson, 1956). In the immature mouse the use of colchicine indicates that fully differentiated typical Leydig cells can and do divide under physiological conditions: this does not appear to take place to any observable extent in the normal adult mouse Leydig cell. Studies on senescent mice have yet to be made.

From Text-fig. 5 it will be seen that the logarithm of the Leydig tissue volume (as calculated from Sudan black sections) undergoes a uniform regular increase of $14{\cdot}5\% \pm 1\%$ per day between birth and the end of the fifth week. In other words during the prepubertal period the absolute volume of Leydig tissue increases at a compound rate of roughly 15% per day. If this daily increment is due to formation of new Leydig cells, then one might expect daily, for every 100 existing cells either fifteen transitions from mesenchyme or fifteen mitotic figures. Since the average daily mitotic rate during the first 5 weeks of postnatal life is $6{\cdot}3\%$, the increase in the Leydig tissue volume is due both to Leydig cell division and to recruitment from mesenchyme in approximately equal proportions, provided that existing morphologically mature Leydig cells do not materially increase in size: if cell size does increase then the mesenchymal contribution must be proportionately reduced.

SUMMARY

1. The testes of thirty-three albino mice, aged from 0 days to 8 weeks, have been examined at 7-day intervals: stains used include H. & E., the PAS reaction, Sudan black, 2:4-dinitrophenyl hydrazine, the plasmal reaction, a mitochondrial stain and an alkaline phosphatase technique. Seminal vesicles were also examined using H. & E. and the alkaline phosphatase technique: seminal vesicle weight has been taken as an indicator of androgen production.

2. The concept of separate foetal and pubertal generations of Leydig cells is thought to be based on inadequate histological methods for demonstrating the relatively slowly growing Leydig tissues in a rapidly expanding prepubertal testis and is erroneous in the mouse at least.

3. The mouse Leydig cell, in common with those of other homoiothermal vertebrates, contains glycoprotein; in contrast to the Leydig cell of poikilotherms it has no glycogen.

4. The mouse Leydig cell has sudanophilic lipids; lipids stainable with 2:4-dinitrophenyl hydrazine and Schiff's reagent are absent from the neonatal Leydig cell, present in large quantities in the prepubertal Leydig cell and present in reduced amounts in the adult cell.

5. Cytomorphosis of the Leydig cell from its mesenchymal precursor includes acquisition of sudanophilic lipids, Schiff stainable lipids and mitochondria.

6. Graphic representation of the growth rates of the Leydig tissue and the seminal vesicle shows that both tissues grow at a similar rate and that the growth of Leydig tissue antedates the growth of the seminal vesicles.

7. The distribution of plasmalogens has been described and is thought possibly to be related to the varying balance between the production of androgen from cholesterol and acetate and its release into the blood stream.

8. Mitotic figures have been demonstrated in typical Leydig cells. The absolute volume of Leydig tissue during the prepubertal phase increases at a compound rate of about 15 % per day: the Leydig mitotic rate is 6·3 % per day. The increment in Leydig tissue volume is thus due to cell division plus recruitment from mesenchyme. Leydig cells in the adult testis do not appear to undergo mitosis in physiological circumstances.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Neonatal testis, H. & E. Note the abundant eosinophilic Leydig cytoplasm. ×350.

Fig. 2. Testis at 7 weeks, PAS. Epithelioid Leydig cells stand out prominently in the intertubular spaces: the extracellular spaces contain PAS positive ground substance. ×150.

Fig. 3. Neonatal testis, Sudan black. Note relative prominence of Leydig tissue. ×90.

Fig. 4. Testis at 7 days, Sudan black. The intensity of Leydig sudanophilia has increased, but the amount is relatively reduced. ×90.

PLATE 2

Fig. 5. Testis at 6 weeks, Sudan black. There is a relative increase in sudanophilic Leydig tissue. ×90.

Fig. 6. Testis at 7 days, the plasmal reaction. Interstitial plasmalogens have just appeared. ×90.

Fig. 7. Testis at 6 weeks, the plasmal reaction. Note the prominence of Leydig cell plasmalogens.

Fig. 8. Adult testis, the plasmal reaction. Leydig plasmalogens are greatly reduced; Sertoli plasmalogens have now appeared. ×90.

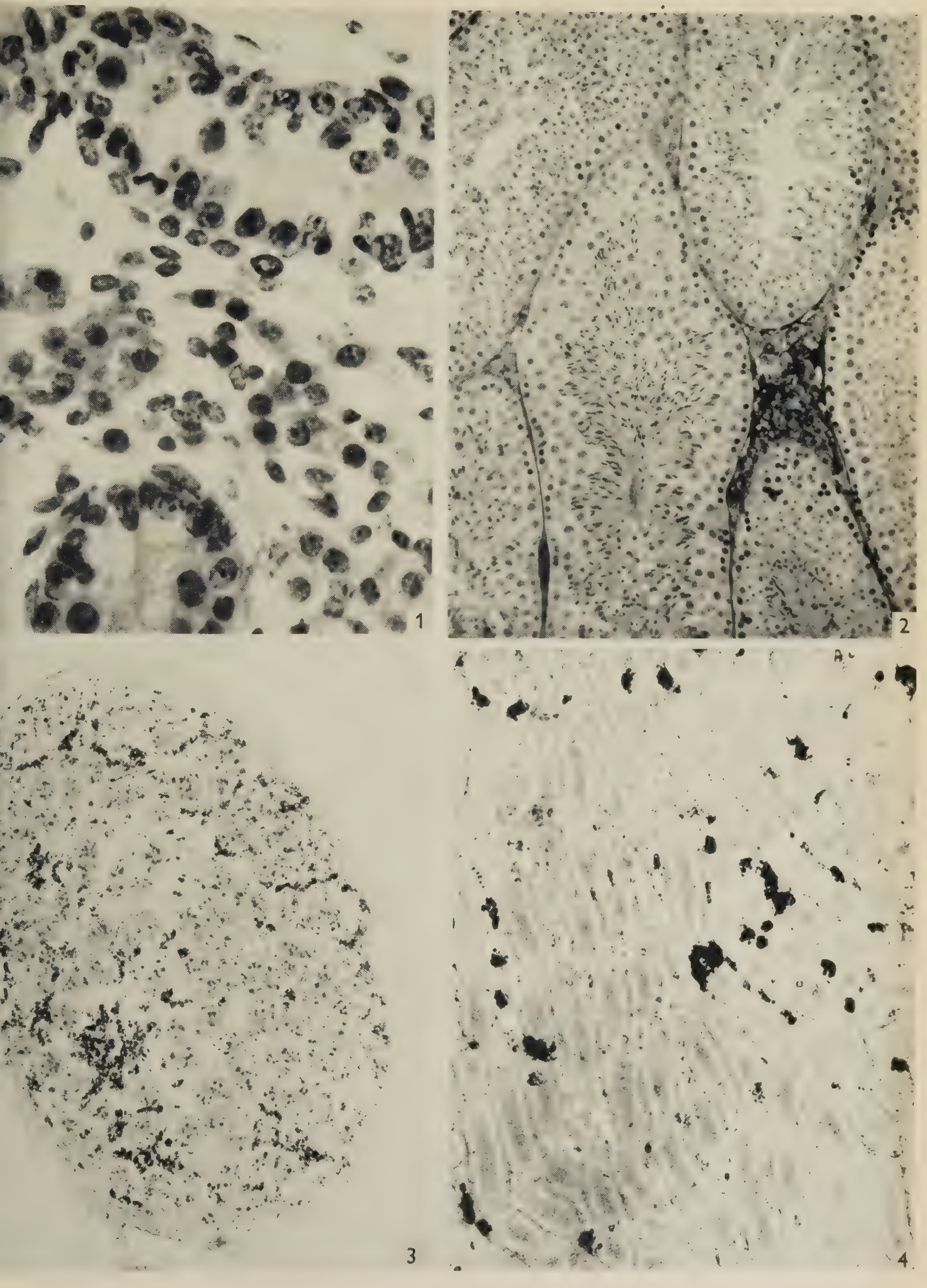
PLATE 3

Fig. 9. Testis at 6 weeks, alkaline phosphatase. The basement membranes of the seminiferous tubules are prominently stained. Note the gross interstitial shrinkage produced by alcoholic fixation. Leydig tissue contains no alkaline phosphatase. ×150.

Fig. 10. Neonatal testis, acid fuchsin and toluidine blue. Leydig cells contain numerous granular mitochondria. ×950.

Fig. 11. Seminal vesicle at 4 weeks, alkaline phosphatase. The stroma contains a large amount of the enzyme. ×150.

Figs. 12 and 13. Testis at 3 weeks, PAS. Colchicine arrested Leydig mitoses are readily visible. ×950.



BAILLIE—Growth and histochemistry of the Leydig tissue

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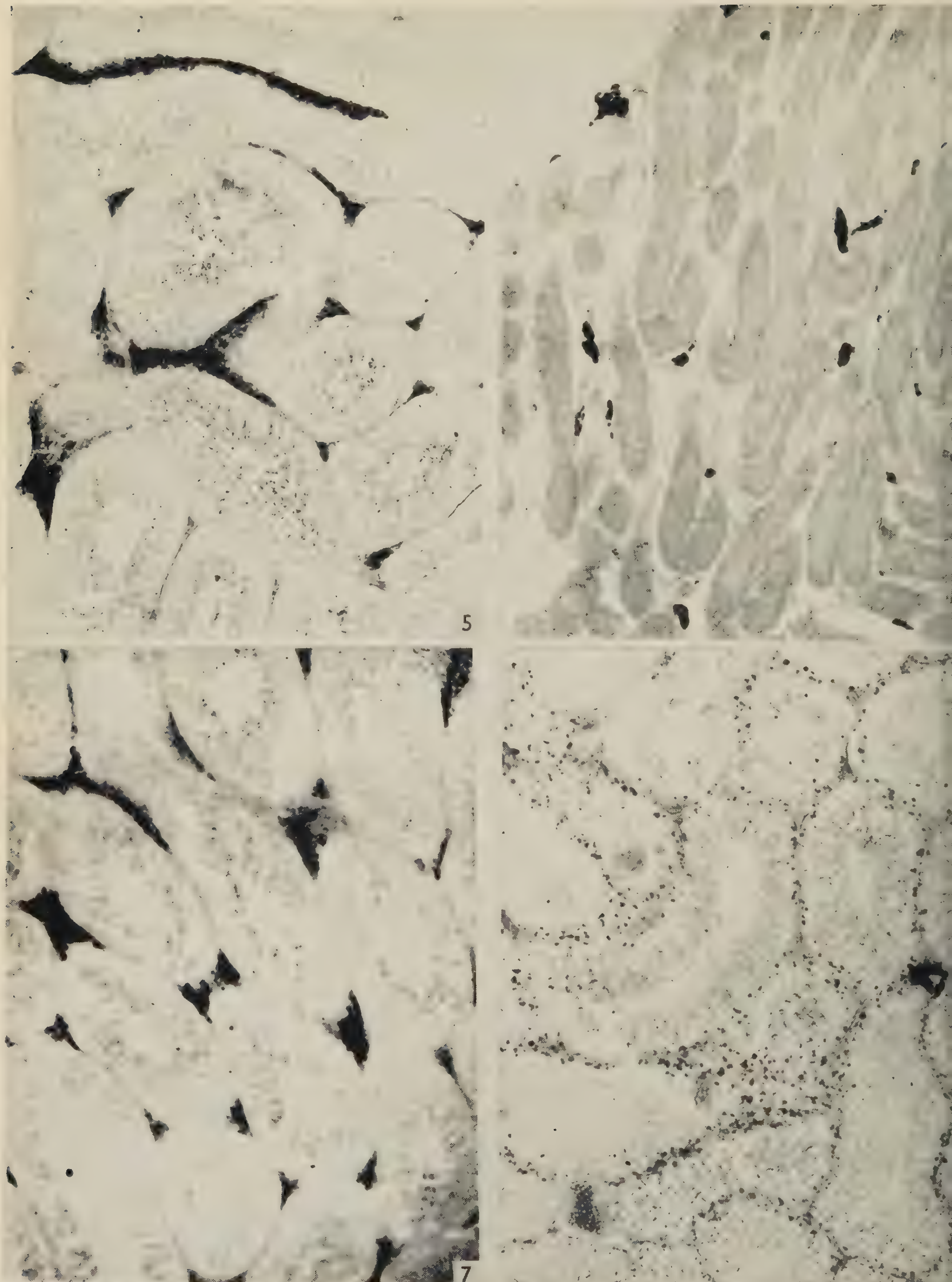
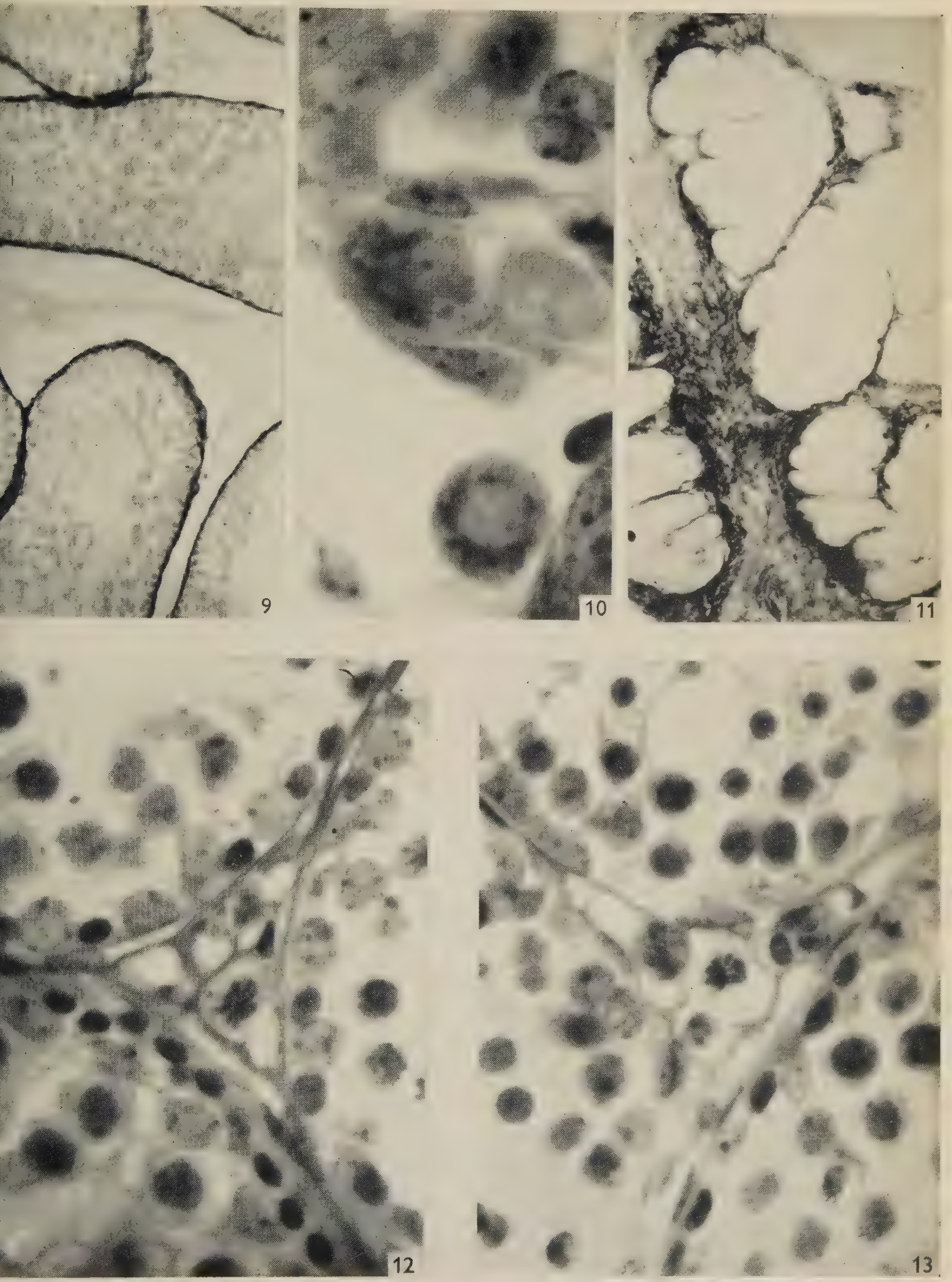


BAILLIE—GROWTH AND HISTOCHEMISTRY OF THE LEYDIG TISSUE



BAILLIE—GROWTH AND HISTOCHEMISTRY OF THE LEYDIG TISSUE

THE NERVE SUPPLY TO THE NICTITATING MEMBRANE OF THE CAT*

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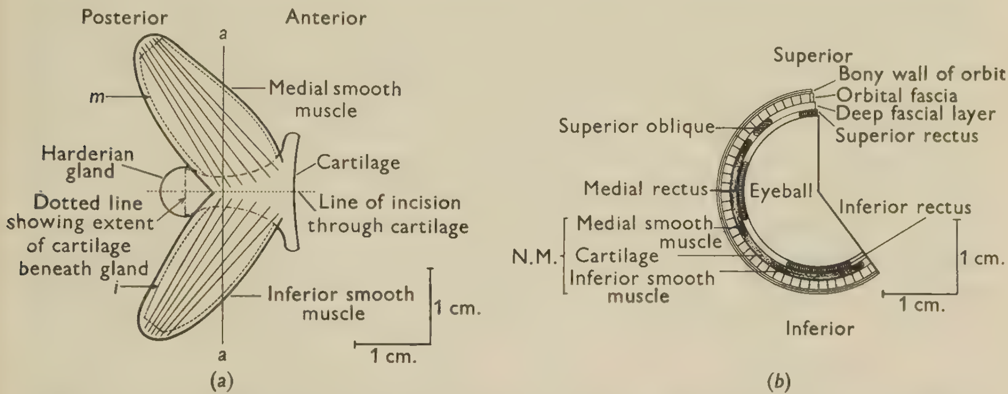
INTRODUCTION

The nictitating membrane of the cat is commonly used to study sympathetic transmission, but the pathways of the sympathetic nerves that innervate the membrane have not been described before. The development of a method whereby the membrane can be set up as an isolated preparation (Thompson, 1955, 1958) has provided an opportunity for the study of its nerve supply.

The membrane contains two thin sheets of smooth muscle. Acheson (1938) showed that these smooth muscles, which he named the medial and inferior muscles,



Text-fig. 1. (*a*) Diagram showing the arrangement of the cartilage, Harderian gland and smooth muscles of the cat's left nictitating membrane, as seen from the medial aspect when laid out flat. Dotted lines give the approximate areas of cartilage and muscle used when setting up the medial (*m*) or inferior (*i*) muscles as isolated preparations (Thompson, 1958). (*b*) Diagram showing the main anatomical relationships of the nictitating membrane in a coronal section through part of the cat's left orbit, viewed from in front. Both diagrams are at the same scale; the approximate level at which the coronal section would pass through the membrane in (*a*) is shown by the line *a*——*a*.

arise deeply in the orbit from the fascial coverings of the medial and inferior recti muscles, respectively, to be inserted into adjacent sides of a T-shaped piece of cartilage (Text-fig. 1*a*, *b*). A method was also devised for using the medial muscle as an isolated nerve-smooth muscle preparation (Thompson, 1957, 1960). The nerve appeared to be a branch of one of the orbital nerves. There was no information about the nerve supply to the other (inferior) smooth muscle. This paper describes the pathways and connexions of the sympathetic nerves to the membrane.

* The work reported in this paper forms part of a Thesis accepted by the University of London for the degree of Ph.D.

METHODS

(1) *The preservation of specimens for dissection*

Adult cats of both sexes were killed by blowing air into the right femoral vein. Both common carotid arteries were ligated in the neck and an arterial cannula was inserted into one of them through which formol-saline solution was allowed to run until there was an obvious stiffening of the tissues of the head and neck. The carotid artery was tied, the head was removed from the animal and stored in formol-saline for at least 3 days before dissection to ensure full hardening of the nervous tissues within the skull. During dissection the exposed areas were kept moist with formol-saline solution in order to prevent drying and shrinking of the tissues. A total of 12 dissections was made.

(2) *Methods used in studying the nerve supply to the membrane in the live animal*

Each of five cats was anaesthetized with ethyl chloride and ether and then prepared as a spinal animal, using the modification of the Barger & Dale (1910) technique described by Kosterlitz, Krayer & Matallana (1955). Spinal animals were used because earlier work had shown that isolated preparations obtained from cats which had received a long-acting general anaesthetic, such as chloralose, were less sensitive than those which had been removed from spinal cats (Thompson, 1958). Although these muscles were to be studied *in vivo*, the same procedure that had been adopted for the *in vitro* experiments was also used here.

In three cats, the eyeball was first removed from one or other orbit as described in an earlier paper (Thompson, 1958) and both the medial and inferior smooth muscles were located and mobilized. From this point on the dissection differed from the original description: the nerve to the medial smooth muscle was found and two fine silk ligatures were passed underneath it and left in position without tying. The nerve to the inferior muscle was located and treated similarly but, in order to do this, a large amount of bone had to be removed from the lower orbital margin so that a Zeiss dissecting microscope could be used to assist in finding the nerve which is about 0·25 mm. in diameter.

When both nerves had been located, the cat was turned on to its back. The preganglionic cervical sympathetic trunk was separated from the vagus, cut about 2 cm. below the superior cervical ganglion and the distal end drawn into a shielded polythene electrode. The electrode was fixed in position by suturing it into the neck wound.

The cat was turned over again and the head was fixed by binding the jaws to an aluminium bar mounted longitudinally on the side of the operating table. The cartilage of the nictitating membrane was cut in half at right angles to its free edge. The incision was extended posteriorly through the Harderian gland, orbital fat and connective tissue, so that both halves of the membrane were anatomically separated as far as possible. Silk threads were tied through the free edges of each half of the cartilage, passed over separate pulleys and attached to two equal isotonic levers with a 10-fold magnification writing frontally on a smoked drum. Both levers were weighted equally so that they exerted a load of 1–3 g. Throughout the experiments, the tissues were kept moist with warm physiological saline.

The fourth and fifth spinal cats were prepared for intracranial stimulation of certain cranial nerves. First, both common carotid arteries were ligated in order to reduce bleeding during and after removal of the brain. Then the scalp and entire cranial vault (including the tentorium) were removed, exposing the cerebral hemispheres and cerebellum. The dura was opened and the brain removed by lifting it away from the base of the skull, beginning anteriorly. As each pair of cranial nerves came into view, they were cut as close to the brain as possible in order to preserve the maximum length of nerve. The cerebellum was removed and the remainder of the brain cleaned out down to the level of the foramen magnum; the basilar artery was ligated. The dura was stripped off the anterior cranial fossa and the lateral aspects of the middle cranial fossa. The roots of the IIIrd, IVth, Vth and VIth cranial nerves were carefully dissected out and fine silk ligatures were placed round the cut ends so that each could be lifted up and placed across a pair of platinum stimulating electrodes.

In the fourth cat, recordings were made first from the intact membrane and then after bisection, from the upper and lower halves separately. A comparison was made between the effects on the nictitating membrane of stimulating the cervical sympathetic and some of the cranial nerves, and finally the sympathetic was cut intracranially on its course along the floor of the middle cranial fossa, proximal to the level at which it joined the trigeminal nerve (see Text-fig. 2).

In the fifth cat, the preparation of the animal was the same except that both membranes were used. First, recordings were made from the whole left membrane, which was then bisected and recordings were made from each half. On the right side, the membrane was bisected and recordings were made from each half throughout the experiment. The eyes were enucleated before recording began.

Throughout these experiments, nerve stimulation was carried out using supramaximal square wave shocks of 0·5 msec. duration and 10 cyc./sec. frequency, applied through either shielded or unshielded platinum electrodes.

RESULTS

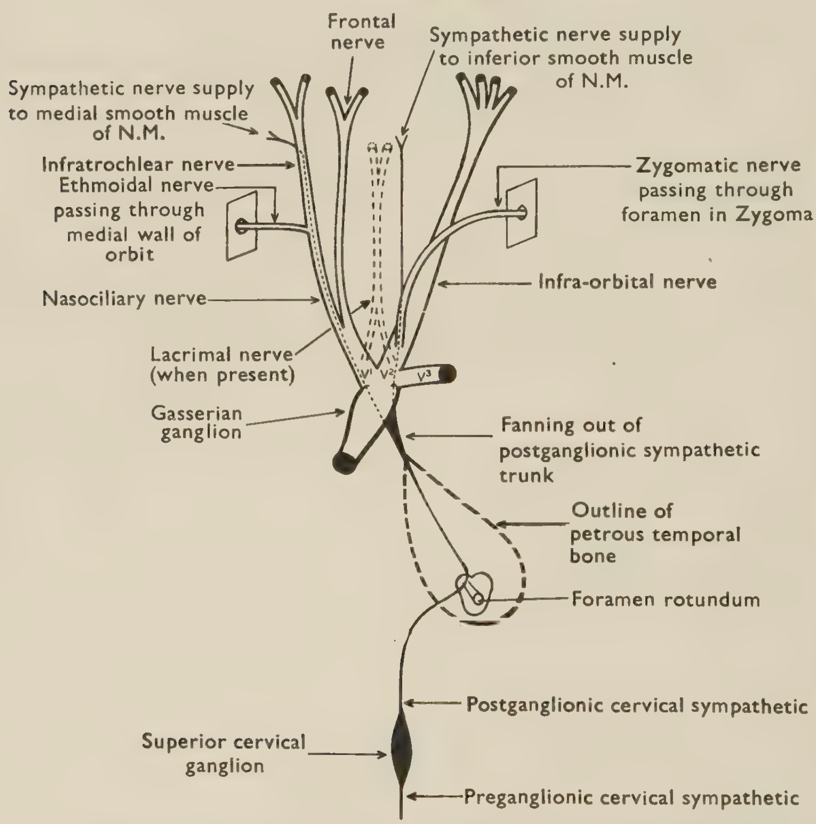
(1) *Anatomical studies*

(*a*) *Sympathetic nerve supply to the medial smooth muscle*

In previous work (Thompson, 1960) it was noted that the sympathetic supply originated from a large nerve which lay on the infero-medial aspect of the superior oblique muscle. Dissections were made in order to identify this nerve.

Pl. 1, fig. 1, shows the appearance of the orbit after removal of the roof, the lateral wall and part of the medial wall. Several nerves are seen, most of them branches of the trigeminal or Vth cranial nerve, whilst the trochlear or IVth cranial nerve supplying the superior oblique muscle is also seen. On the left of the photograph is the Gasserian ganglion which gives rise to the ophthalmic, maxillary and mandibular divisions, each of which subsequently divides into smaller branches. Thus, the ophthalmic division usually divides into the frontal and naso-ciliary nerves, whilst the maxillary division divides into the infra-orbital and zygomatic branches. A diagram showing the important branches of the ophthalmic and maxillary divisions of the trigeminal nerve is given in Text-fig. 2.

The nasociliary nerve divides terminally into ethmoidal and infratrochlear branches; the nasociliary nerve then becomes the infratrochlear nerve (Text-fig. 2). The nerve that lies on the infero-medial aspect of the superior oblique is the infratrochlear (Pl. 1, fig. 1). Pl. 1, fig. 1, shows the origin of the sympathetic supply to the medial smooth muscle: the fine branch which supplies the muscle leaves the infratrochlear nerve about mid-way along its course. At its distal end, the infratrochlear nerve divides into sensory branches which supply the skin on the medial aspect of



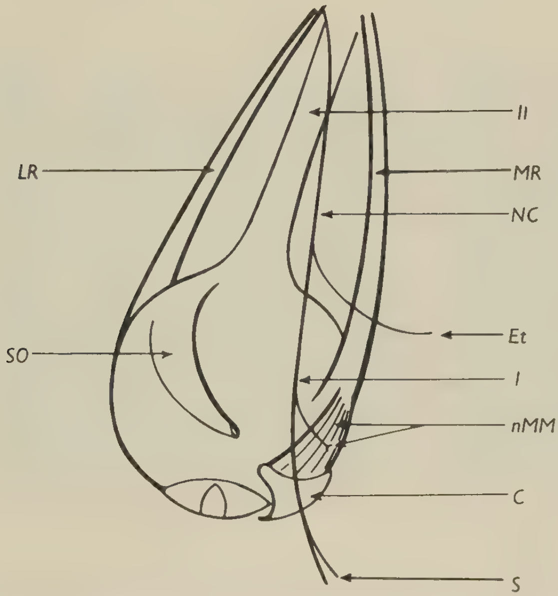
Text-fig. 2. Diagram showing the course of the postganglionic cervical sympathetic nerve supply to the nictitating membrane (*NM*). The pathway of the fibres through the middle ear is shown and the arrangement whereby the fibres gain access to the trigeminal nerve in the middle cranial fossa. The important branches of the ophthalmic (V^1) and maxillary (V^2) divisions are shown, together with their relationship to the postganglionic sympathetic fibres supplying the two smooth muscles of the membrane. Since the lacrimal nerve is not always present, it is shown in dotted outline, as also is its variable origin. V^3 = mandibular division. (The lower part of the diagram is based on Fig. 3 of de Kleijn & Socin, 1915.)

the upper lid near the inner angle (Reighard & Jennings, 1901). These relationships are shown diagrammatically in Text-fig. 3.

The sympathetic supply to the medial smooth muscle leaves the infratrochlear nerve at approximately the level of the main area of origin of this muscle from the

fascial sheath covering the belly of the medial rectus muscle. It is usually a thread-like structure (see Pl. 1, fig. 1) with a diameter of approximately 0·25 mm. After leaving the medial aspect of the infratrochlear nerve, it passes antero-inferiorly for about 3–4 mm., then divides and enters the supero-lateral surface of the medial smooth muscle.

In one specimen the sympathetic supply arose from the ethmoidal nerve and passed directly forwards over the upper border of the medial rectus muscle to reach and supply the medial smooth muscle.



Text-fig. 3. Diagram of the right eye seen from above to show the manner in which the sympathetic nerve supply to the medial smooth muscle (*nMM*) arises from the infratrochlear nerve (*I*). The origin of the medial smooth muscle from the belly of the medial rectus (*MR*) is also shown. *C*, cartilage; *Et*, ethmoidal nerve; *I*, infratrochlear nerve; *LR*, lateral rectus; *MR*, medial rectus; *NC*, nasociliary nerve; *nMM*, nerve supplying medial smooth muscle; *S*, terminal sensory branches of infratrochlear nerve; *SO*, superior oblique; *II*, optic nerve.

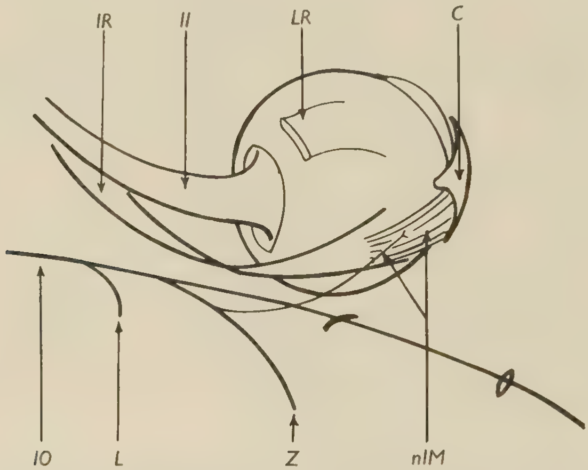
The orbit of the cat usually contains another branch of the trigeminal nerve, the lacrimal nerve, although this is sometimes absent (Windle, 1926) (see Pl. 2, fig. 2). When present, this arises from the ophthalmic division (Mivart, 1881) or from the maxillary division (Reighard & Jennings, 1901) of the trigeminal nerve (see Text-fig. 2).

(*b*) *Sympathetic nerve supply to the inferior smooth muscle*

During the course of repeated dissections in which the inferior smooth muscle was prepared as an isolated preparation (Thompson, 1958) the presence of a recognizable sympathetic nerve supply to the inferior muscle was constantly looked for but never found. No fibres passed from the medial to the inferior muscle, thus ruling out a common source of innervation. Since the sympathetic nerve supply to the medial

muscle was found to be distributed with certain branches of the trigeminal nerve, it seemed possible that the inferior muscle derived its sympathetic nerve supply similarly but independently.

Dissections of the orbit were made and showed that the inferior smooth muscle did, in fact, receive a separate nerve from a branch of the maxillary division. Pl. 1, fig. 2, shows one of these dissections, in which the eyeball was removed and the remaining contents of the orbit rotated medially in order to reveal structures lying beneath the nictitating membrane on the floor of the orbit. All three divisions of the trigeminal nerve can be seen, in which the maxillary division gives off two branches and then passes forwards and laterally in the floor of the orbit as the infra-orbital nerve. The first and larger branch is the lacrimal nerve which passes upwards



Text-fig. 4. Diagram of the right eye seen from the lateral side. The eyeball has been rotated medially in order to show the manner in which the sympathetic nerve supply to the inferior smooth muscle (*nIM*) arises from the zygomatic nerve (*Z*). The origin of the inferior smooth muscle from the belly of the inferior rectus (*IR*) is also shown. *C*, cartilage; *IO*, infra-orbital nerve; *IR*, inferior rectus; L, lacrimal nerve; *LR*, lateral rectus; *nIM*, nerve supplying inferior smooth muscle; *Z*, zygomatic nerve; *II*, optic nerve.

and laterally. The second and smaller branch is the zygomatic nerve, which passes forward and laterally to enter the zygomatic foramen in the frontal process of the zygomatic bone. (In the dissection of the zygomatic branch shown in Pl. 1, fig. 2, the termination of the nerve has been cut just before it enters the zygomatic bone; as a result, the nerve is shortened and is seen to lie freely on the floor of the orbit.)

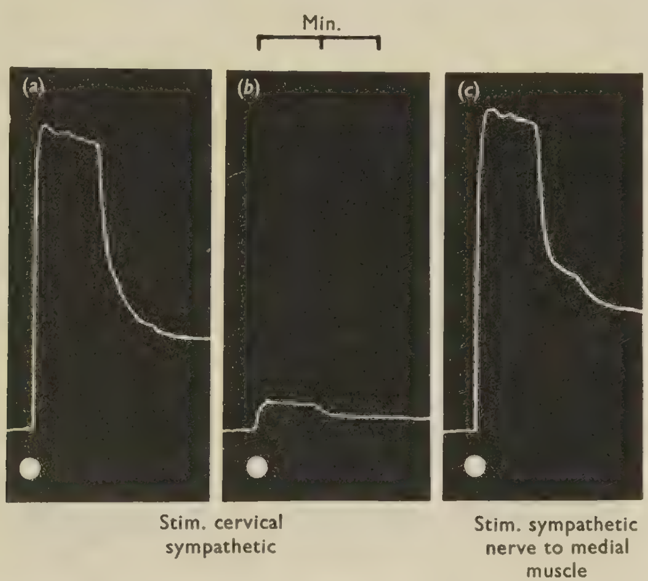
At the point at which the zygomatic nerve changes course, it gives off a fine branch, approximately 0·25 mm. diameter, which passes forwards to enter and supply the inferior smooth muscle (as shown in Pl. 1, fig. 2). This is usually about 1·5 cm. in length and is the sympathetic supply to the inferior muscle; it divides into several minute branches which enter the infero-lateral aspect of the muscle. These relationships are shown diagrammatically in Text-fig. 4.

(2) *Functional studies*

When the dissections had established the course of the nerves which appeared to innervate the nictitating membrane, these nerves were stimulated to find out whether they did so. Experiments were made on five spinal cats to test the following points:

First, that the contractions of the membrane in response to stimulation of the cervical sympathetic nerve supply could be abolished by severing the nerves.

Secondly, that electrical stimulation of the nerves within the orbit would cause contractions of comparable size to those produced on stimulation of the cervical sympathetic.



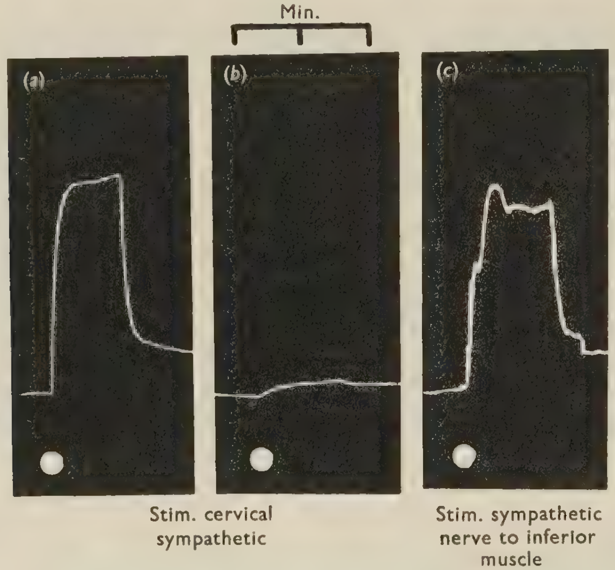
Text-fig. 5. Contractions recorded from upper (medial) half of right nictitating membrane (spinal cat). (*a*) Response obtained on supramaximal stimulation of preganglionic cervical sympathetic at 10 cyc./sec. for 1 min. Between (*a*) and (*b*), the sympathetic supply to the medial smooth muscle was interrupted by cutting the infratrochlear nerve, and in (*b*) the cervical sympathetic stimulation was repeated at 10 cyc./sec. for 1 min.; the response was greatly diminished. In (*c*), the cut distal end of the infratrochlear nerve (containing the postganglionic sympathetic fibres to the medial smooth muscle) was stimulated supramaximally at 10 cyc./sec. for 1 min.; the amplitude of the contraction was actually slightly larger than the control. Time scale = min.

Thirdly, that electrical stimulation of the ophthalmic and maxillary divisions of the trigeminal nerve at sites distal to the levels at which the sympathetic fibres joined them produced contractions as large as those obtained by stimulating the cervical sympathetic.

In addition, the nerves supplying the extraocular muscles were stimulated intracranially in order to exclude the possibility that additional sympathetic fibres travelling with *these* nerves supplied the smooth muscle of the nictitating membrane.

The experiments on bisected membranes were technically difficult to perform and for this reason it was not always possible to obtain results from both halves of the membrane in the same experiment. Text-fig. 5 shows the responses of the upper half of a nictitating membrane from one of these experiments.

The first contraction was that produced on supramaximal stimulation of the sympathetic proximal to the superior cervical ganglion at 10 cyc./sec. The infratrochlear nerve was then cut just proximal to the point at which the branch supplying the medial smooth muscle left it. Stimulation of the cervical sympathetic was repeated. It resulted in a very small contraction, showing that the majority of the sympathetic fibres to the medial smooth muscle had been interrupted. The cut distal end of the infratrochlear nerve was stimulated directly with supramaximal shocks at 10 cyc./sec. and resulted in a contraction of a magnitude comparable to the control response. This confirmed that the bulk of the sympathetic nerve fibres supplying the medial smooth muscle were carried in the trunk of the infratrochlear nerve. Three experiments of this kind gave similar results.



Text-fig. 6. Contractions recorded from lower (inferior) half of right nictitating membrane (spinal cat). (*a*) Response obtained on supramaximal stimulation of preganglionic cervical sympathetic at 10 cyc./sec. for 1 min. Between (*a*) and (*b*) the sympathetic nerve to the inferior smooth muscle was cut and in (*b*), the preganglionic cervical sympathetic trunk was again stimulated at 10 cyc./sec. for 1 min.; the response was almost completely abolished. In (*c*), the cut distal end of the sympathetic nerve to the inferior smooth muscle was stimulated supramaximally at 10 cyc./sec. for 1 min.; the amplitude of the contraction was almost the same as that of the control, the irregularities being due to the unavoidable stimulation of neighbouring striated muscles in the floor of the orbit. Time scale = min.

Another three experiments were performed on the nerve supply to the lower half of the membrane. Text-fig. 6 shows the recording obtained from one of them. The contraction due to stimulation of the cervical sympathetic at 10 cyc./sec. was shown first. The sympathetic nerve to the inferior muscle was then cut and when stimulation was repeated the response was minute. Finally, the distal cut end was stimulated with supramaximal square waves applied through platinum electrodes and the resulting contractions were as large as those obtained originally. These results confirmed that the nerve which had been cut did, in fact, carry the bulk of

the intracranial portions of the sympathetic nerve fibres to the inferior smooth muscle.

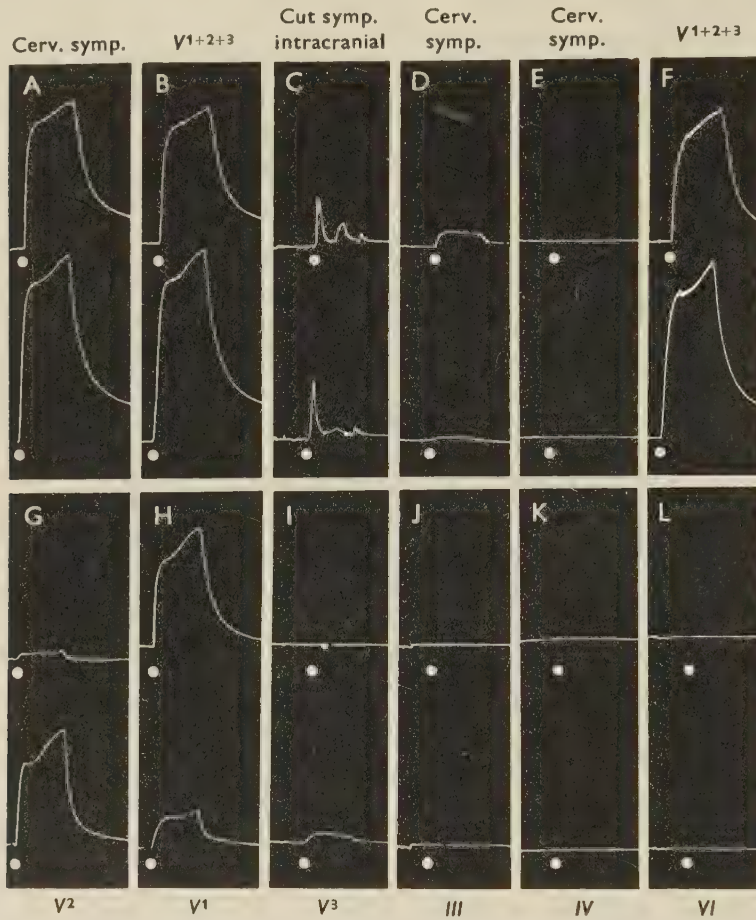
In one animal, recordings were first made from both halves of one bisected membrane. The sympathetic nerves to the medial and inferior smooth muscles were then cut. The animal was killed and the head removed and preserved for dissection. After fixation in formalin the orbit was dissected in order to check that the nerves which were cut during the live experiment were the same as those which in earlier dissections were considered to supply the medial and inferior smooth muscles. Photographs of this dissection are shown in Pl. 2, figs. 1 and 2, from which it can be seen that the nerves were indeed the same.

The results indicated that the two smooth muscles of the nictitating membrane were supplied by two separate nerves which were composed of postganglionic fibres that used certain branches of the trigeminal nerve for their distribution. It now remained to confirm this by means of stimulating the appropriate divisions of the trigeminal nerve intracranially. In addition, the oculomotor, trochlear and abducens cranial nerves were stimulated in order to check that these nerves were not carrying fibres to the membrane.

In three experiments on spinal cats the brain was removed and the intracranial nerve roots were stimulated electrically. The membrane was bisected as before but the eyeball was left intact, except in one experiment in which it was enucleated. Text-fig. 7 shows typical results from one experiment.

First, the contractions of both halves of the membrane in response to stimulation of the cervical sympathetic with supramaximal shocks at 10 cyc./sec. were shown (Text-fig. 7A). The recordings from the upper and lower halves of the membrane were independent because the lever did not move when the opposite half of the membrane was moved with a pair of forceps. A pair of platinum electrodes was then straddled across all three divisions of the trigeminal nerve. Supramaximal stimulation at 10 cyc./sec. then produced contractions of both halves which were practically the same size as those produced on stimulation of the cervical sympathetic (Text-fig. 7B). The postganglionic trunk was found to pass forwards from the apex of the petrous temporal bone over the floor of the middle cranial fossa to join the various cranial nerves, an observation made earlier by Barlow & Root (1949). The postganglionic trunk was cut in a coronal plane along a line extending from the middle of the pituitary fossa to just posterior of the foramen ovale (Text-fig. 7C). The cervical sympathetic was stimulated (Text-fig. 7D); the nerve was cut again to complete its transection; stimulation then failed to produce any response (Text-fig. 7E). This showed that all the postganglionic fibres had been severed. On cutting the postganglionic sympathetic in the middle cranial fossa, it was noted that the more medial fibres supplied the medial muscle whilst the more lateral fibres supplied the inferior muscle. The three divisions of the trigeminal nerve were separated by dissection and stimulated singly. Text-fig. 7H showed that stimulation of the first division (V^1) resulted in a large contraction of the upper half of the membrane but a small contraction of the lower half. By contrast, stimulation of the second division (V^2) (Text-fig. 7G) produced the opposite effect. Stimulation of the third division (V^3) (Text-fig. 7I) resulted in a small contraction of the lower half and had no effect on the upper half. These results again confirmed that the postganglionic sympathetic

nerve supply to the medial and inferior muscles travelled in branches of the first and second divisions. The small contraction of the lower half of the membrane which was produced as a result of stimulating the mandibular division indicated that a few sympathetic fibres to the inferior muscle were present in the mandibular division.



Text-fig. 7. Contractions of the upper and lower halves of a right bisected nictitating membrane. Upper tracing = upper (medial) half and lower tracing = lower (inferior) half of membrane. Brain removed; eye enucleated. Supramaximal nerve stimulation at 10 cyc./sec. for 1·5 min., throughout. (A) Stimulation of preganglionic cervical sympathetic trunk. (B) Combined stimulation of ophthalmic (V^1), maxillary (V^2) and mandibular (V^3) divisions of trigeminal nerve. (C) Postganglionic cervical sympathetic trunk cut in middle cranial fossa proximal to level at which fibres joined trigeminal nerve. Small contractions seen are due to stimulation of postganglionic nerve fibres on cutting them. (D) Stimulation of preganglionic cervical sympathetic trunk produced small residual responses of both halves of the membrane, showing that a few fibres had been left intact. Postganglionic trunk in middle cranial fossa again cut and in (E) cervical sympathetic restimulated with no effect, showing that all fibres had now been severed. (F) V^{1+2+3} stimulated. The divisions were then stimulated separately; (G) V^2 stimulated, (H) V^1 stimulated, and (I) V^3 stimulated. Lastly, the nerves to the extra-ocular muscles were stimulated (J) *III*, (K) *IV* and (L) *VI*, producing no effect on either half of the membrane.

Subsequently, these fibres travelled to the inferior muscle by some unknown route.

In three experiments, the cranial nerves supplying the extraocular muscles (III, IV and VI) were stimulated. In none did the nictitating membrane contract. These stimulations did not bring about contraction of the membrane whether the eyeball was present or had been removed. Furthermore, the extra-ocular muscles moved little which may be attributed to their poor blood supply; for before the brain was removed, both carotid arteries and the basilar artery had been ligated. Although this did not appear to depress the functioning of the nictitating membrane nor of its nerve supply, the functioning of the striated extra-ocular muscles may have been impaired.

DISCUSSION

In spite of the extensive use of the cat's nictitating membrane in investigations on the sympathetic nervous system, only de Kleijn and Socin (1915) have attempted to trace the course of the postganglionic sympathetic nerve supply to the membrane. As long ago as 1727 Francois Pourfour du Petit established that the muscle activating the membrane in the dog is innervated by the cervical sympathetic. Langley (1900) showed that preganglionic fibres from the first five thoracic nerves synapse with cells within the superior cervical ganglion which supply postganglionic fibres to the nictitating membrane of the cat. De Kleijn & Socin (1915) carried out a beautiful study of the course of the cervical sympathetic in the cat, following it from its point of entry into the skull to its arrival in the middle cranial fossa. They plotted the course of the fibres. They showed that the postganglionic fibres enter the skull by passing through the carotid canal with the internal carotid artery. For this reason the postganglionic sympathetic trunk is usually named the internal carotid nerve by anatomists (see, for example, Ranson & Billingsley, 1918). The fibres then deviate laterally and pass through the middle ear, coursing across the base of the promontory. From here they enter the bone of the base of the skull, pass antero-medially and according to de Kleijn & Socin (1915), enter the cranial cavity by passing through the bone just medial to the foramen rotundum, but lateral to the foramen for the Vidian nerve. After this, the sympathetic fibres of the pupil pass by way of the ophthalmic division of the trigeminal nerve and the long ciliary nerves. The postganglionic sympathetic fibres do not pass through the Gasserian ganglion, but unite with the ophthalmic division of the trigeminal nerve beyond the ganglion. De Kleijn & Socin (1915) were never able to identify the pathway of the sympathetic fibres to the nictitating membrane and the eyelid.

The passage of the sympathetic fibres through the middle ear has received a great deal of attention. For instance, Francois-Franck (1878–79) noted that stimulation of some of the nerve fibres which pass through the middle ear of the dog produced dilatation of the pupil. Although he did not mention whether this procedure had any effects on the nictitating membrane he did observe that the dilatation of the pupil induced by stimulation of the cervical sympathetic could be abolished if he cut the ophthalmic division of the trigeminal nerve in front of, but not behind, the Gasserian ganglion. Jarcho & Root (1940) showed that the postganglionic fibres to the cat's nictitating membrane must pass through the middle ear because labyrinthectomy is followed by an increased sensitivity of the membrane to I.V. adrenaline,

similar to that which develops after the cervical sympathetic is cut. Many others have shown that the postganglionic cervical sympathetic fibres pass through the middle ear in different species, including man (Loewenfeld, 1958).

The results of the present experiments indicate that the postganglionic sympathetic fibres supplying the nictitating membrane of the cat travel with branches of the trigeminal nerve. The picture is more complicated than de Kleijn & Socin (1915) could have foreseen, since they were unaware that the membrane was moved by two smooth muscles (Acheson, 1938). These muscles, called medial and inferior, receive separate innervations via different divisions of the trigeminal nerve. The medial muscle is supplied by fibres which run with the nasociliary and then the infratrochlear branches of the ophthalmic division. The inferior muscle is supplied by fibres which run with the infra-orbital and zygomatic branches of the maxillary division. The findings explain the observation of E. S. Perkins (personal communication) that cutting the nasociliary nerve produced a partial paralysis of the cat's nictitating membrane.

The postganglionic fibres enter the cranial cavity by passing between the articulations of the petrous part of the temporal and alisphenoid bones (Barlow & Root, 1949). The fibres then pass anteriorly, fan out, and enter the trigeminal nerve as it passes forwards in the middle cranial fossa dividing into ophthalmic, maxillary and mandibular contributions. De Kleijn & Socin (1915) stated that the fibres to the nictitating membrane passed separately from those for the pupil. The present experiments have shown that the fibres supplying the nictitating membrane and those supplying the pupil travel together, in disagreement with de Kleijn & Socin but in agreement with Barlow & Root and with Francois-Franck.

When the cervical sympathetic is stimulated the nictitating membrane contracts; cutting the sympathetic supply close to the membrane does not always abolish the contractions, whereas cutting the supply in the middle cranial fossa does. This shows that some sympathetic fibres reach the membrane by other routes. There are two possibilities: either they pass for part of the way with the branches of the trigeminal nerve but leave them more proximally or they run quite independently. If this is so, the fibres might run with another cranial nerve in the orbit or on their own.

The present experiments provide no evidence that some postganglionic fibres to the nictitating membrane accompany the cranial nerves which supply the extra-ocular muscles. However, the oculomotor, trochlear and abducens nerves were not stimulated *within the orbit* and therefore the possibility has not been excluded that sympathetic fibres destined for the membrane may join these cranial nerves within the orbit. It seems most unlikely that they do so because de Kleijn & Socin removed the four recti and two oblique eye muscles together with their nerves, the eyeball and optic nerve, the long and short ciliary nerves and the ciliary ganglion, without abolishing the effect of cervical sympathetic stimulation on the nictitating membrane.

De Kleijn & Socin also stated that they were able to remove all three trigeminal rami without any adverse effect on the contractions of the membrane. These findings appear to be at variance with the present results; but since the authors do not state how extensive their removal of the rami was, it is possible that enough of the first and second rami remained to preserve the point of entry of the sympathetic fibres. Barlow & Root (1949) noted also that intracranial stimulation of the ophthalmic

division of the trigeminal nerve produced not only dilatation of the pupil but also retraction of the nictitating membrane. Although they also stimulated the maxillary and mandibular divisions they made no mention of any effects on the nictitating membrane.

In vivo movements of the nictitating membrane may be produced as a result of anatomical connexions between it and certain of the external ocular muscles (Rosenblueth & Bard, 1932; Paton & Thompson, 1953). These effects can be reproduced experimentally by stimulating the cranial nerves supplying the muscles, and Rosenblueth & Bard came to the conclusion that contractions of the lateral rectus muscle produced most movement of the membrane. Since the present experiments on the membrane were usually done with the eyeball enucleated or eviscerated, the mechanical effects of contractions of the external ocular muscles cannot be compared with those occurring in the presence of an intact eyeball. There is therefore no disagreement between these results and those obtained earlier. Rosenblueth & Bard (1932) stimulated the ophthalmic division and reported that it produced 'variable effects which may be interpreted as reflex'. Bennati & Isola (1946) claimed that stimulation of the cervical sympathetic trunk in the cat and dog caused a contraction of the superior oblique muscle of the eye. Brown (1951) observed that the 'contraction' of the superior oblique disappeared if the muscle was freed from its connexion with the nictitating membrane.

SUMMARY

1. In cats, the postganglionic sympathetic nerves to the smooth muscles of the nictitating membrane were traced by twelve dissections of the orbit and cranial cavity. The results were confirmed by stimulating the nerves so demonstrated in five spinal cats.

2. The medial and inferior smooth muscles are supplied by separate postganglionic sympathetic nerves which gain access to the muscles by accompanying branches of the trigeminal nerve.

3. The postganglionic sympathetic nerve fibres enter the cranial cavity beneath the apex of the petrous temporal bone and pass anteriorly and medially to join the trigeminal nerve on its inferior surface in the floor of the middle cranial fossa.

4. The sympathetic supply to the medial muscle accompanies first the ophthalmic division and then its nasociliary and infratrochlear branches. Most of the fibres leave the infratrochlear nerve together and enter the muscle as a fine branch; a few fibres enter the muscle independently, probably by leaving the nerve more proximally.

5. The sympathetic supply to the inferior muscle accompanies first the maxillary division and then its zygomatic branch. Most of the fibres leave the zygomatic nerve together, entering the muscle as a fine branch; a few enter the muscle independently, probably by leaving the nerve more proximally.

6. There was no evidence to suggest that the oculomotor (III), trochlear (IV) or abducens (VI) nerves carried any postganglionic sympathetic fibres to the nictitating membrane. The results support earlier conclusions that, *in vivo*, movements of the nictitating membrane which accompany contractions of the extra-ocular muscles are due to anatomical connexions between them.

The author wishes to thank Prof. W. D. M. Paton, F.R.S., for all his stimulating discussion during these experiments and Prof. G. V. R. Born and Dr J. R. Vane for their helpful criticism during the preparation of this paper. He would also like to thank Mr D. A. Green and staff for technical assistance and Miss Yvette Galvin for her painstaking secretarial work.

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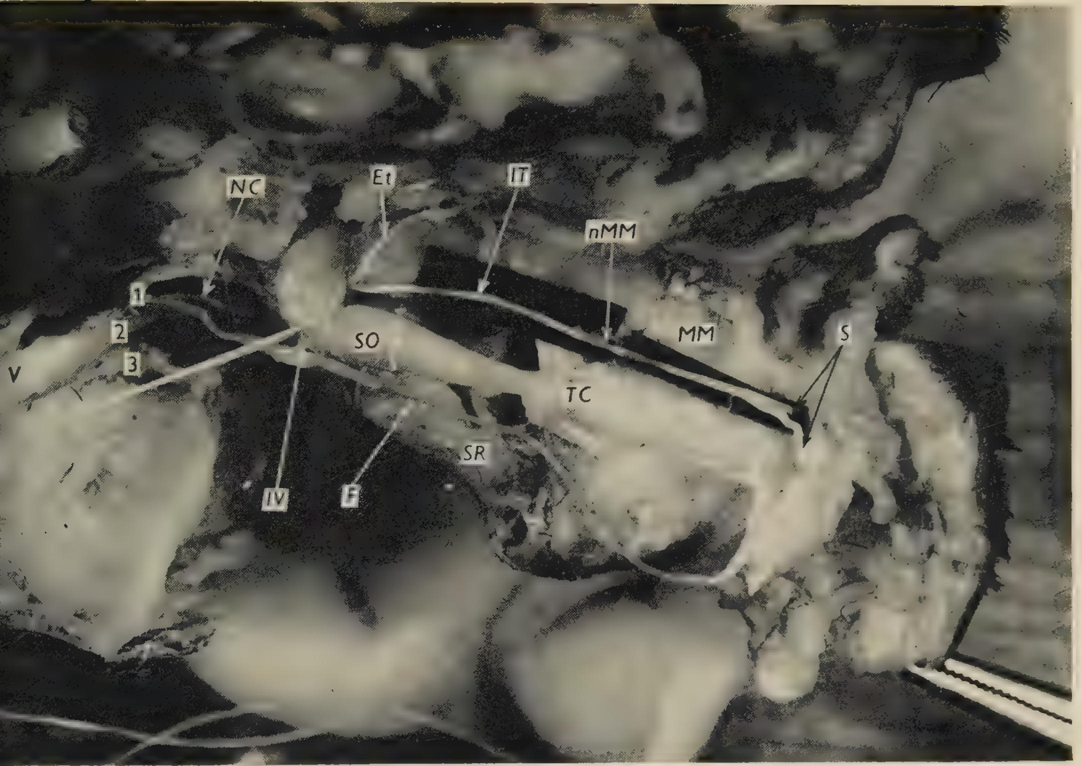

Fig. 1

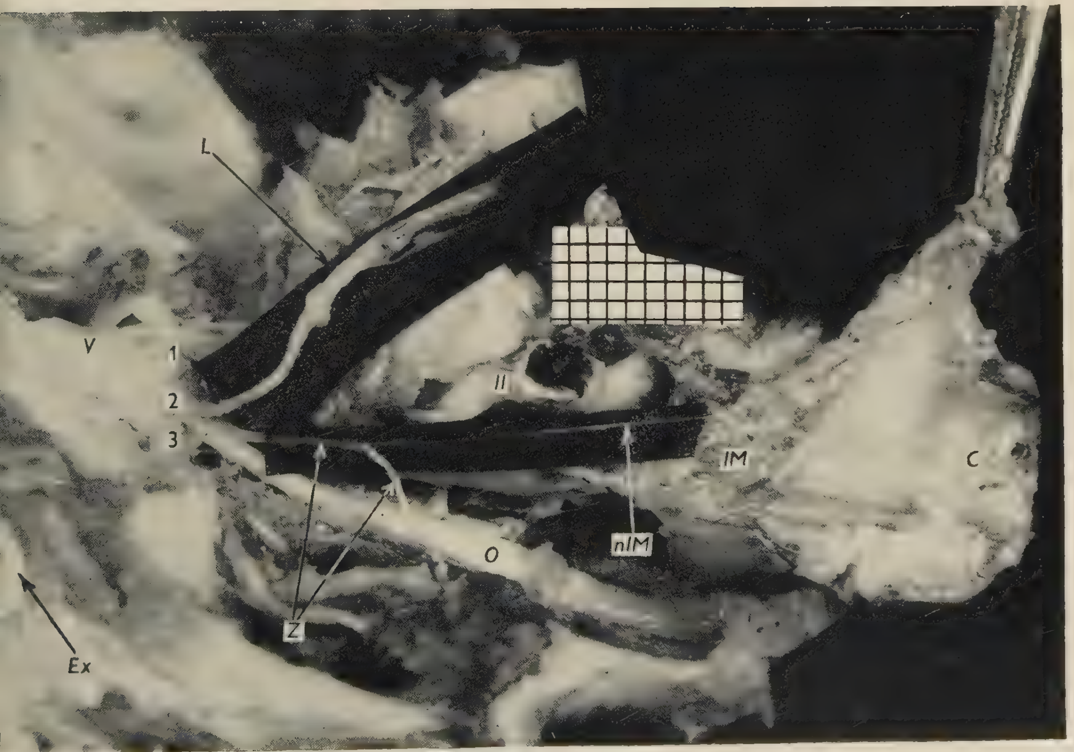

Fig. 2

THOMPSON—NERVE TO NICTITATING MEMBRANE

(*Facing p. 384*)

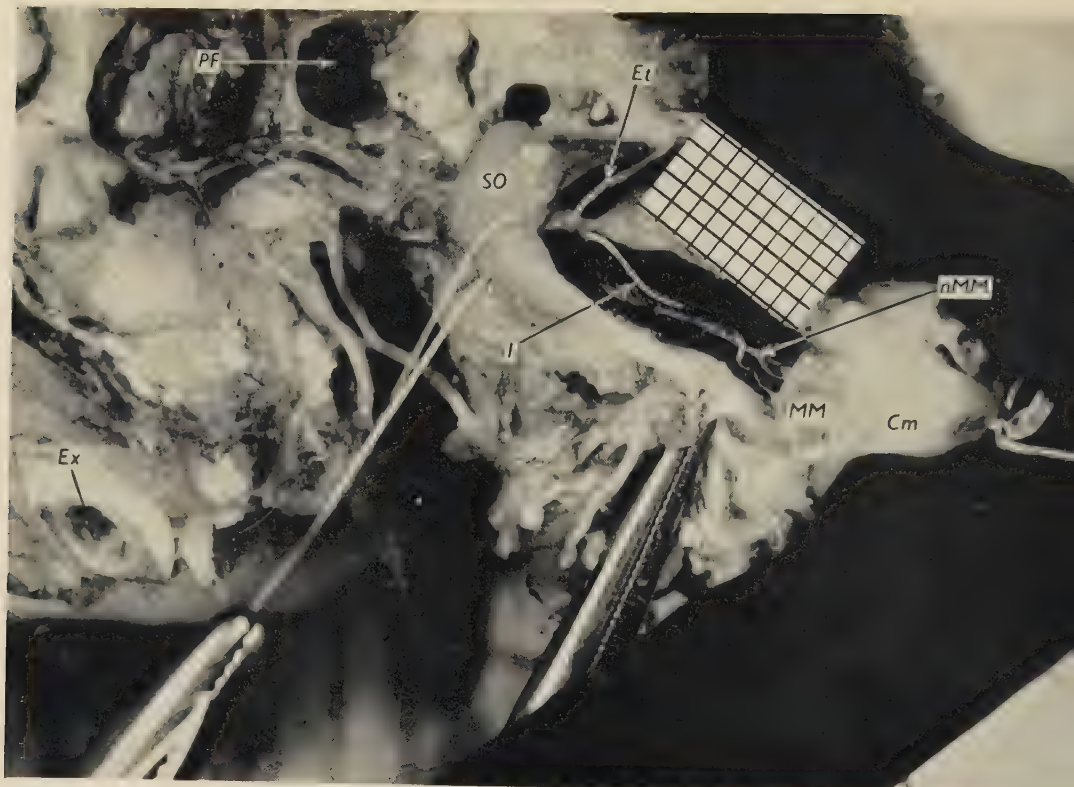

Fig. 1

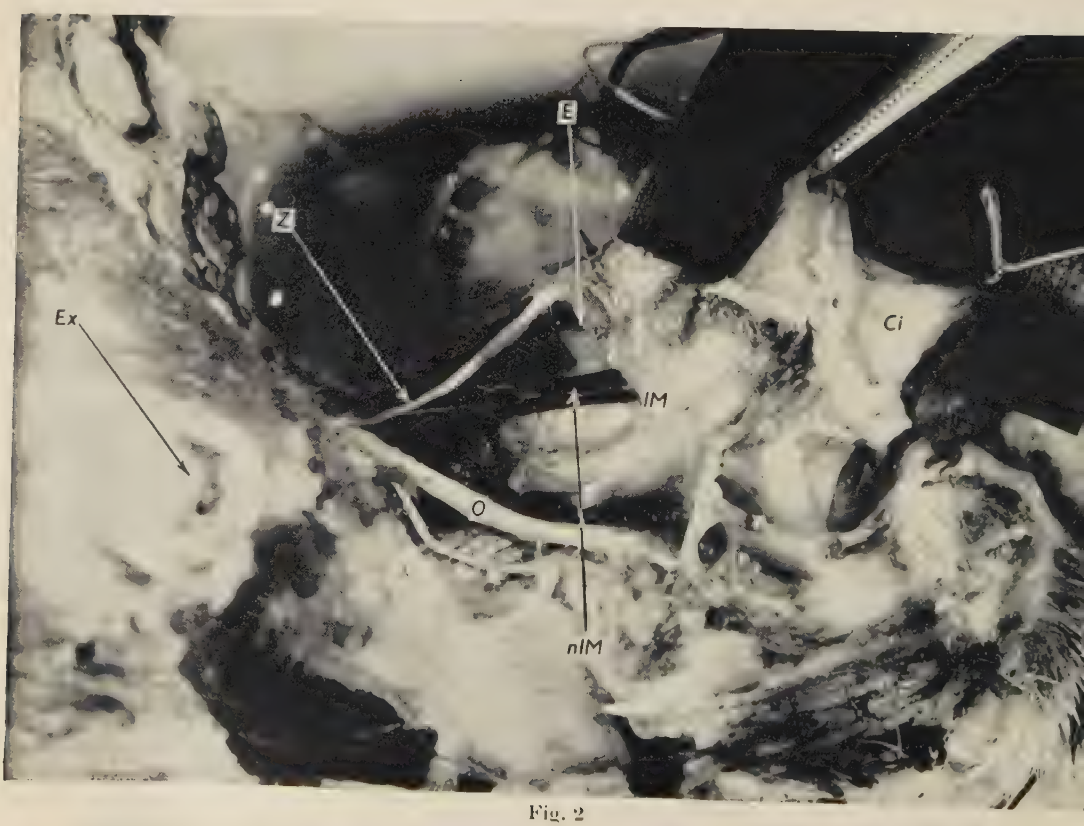

Fig. 2

EXPLANATION OF PLATES

All are photographs of dissections of the preserved right orbits of two cats, in which the roof and walls of the orbit were removed. Small strips of black paper were placed behind some structures in order to improve contrast.

Plate 2, figs. 1 and 2 are photographs of the right orbit of a spinal cat in which the sympathetic nerves to the medial and inferior smooth muscles were first identified by electrical stimulation. (For details, see text.)

Abbreviations used

C, cartilage of nictitating membrane; *Ci*, half of cartilage associated with inferior muscle; *Cm*, half of cartilage associated with medial smooth muscle; *E*, eyeball; *Et*, ethmoidal nerve; *Ex*, external ear; *F*, frontal nerve; *I*, infratrochlear nerve; *IM*, inferior smooth muscle; *L*, lacrimal nerve; *MM*, medial smooth muscle; *NC*, nasociliary nerve; *nIM*, sympathetic nerve to the inferior smooth muscle; *nMM*, sympathetic nerve to the medial smooth muscle; *O*, infra-orbital nerve; *PF*, pituitary fossa; *S*, sensory branches; *SO*, superior oblique; *SR*, superior rectus; *TC*, trochlear cartilage; *II*, optic nerve; *N*, trochlear nerve; *V*, trigeminal nerve and its (1), ophthalmic, (2), maxillary, and (3), mandibular divisions.

Plate 1

Fig. 1. Structures arranged to show sympathetic nerve supply to medial smooth muscle. The trigeminal nerve (*V*) can be seen giving rise to ophthalmic (1), maxillary (2), and mandibular (3), divisions. The ophthalmic division (1), first divides into frontal (*F*), and nasociliary (*NC*), branches, the latter then redividing into ethmoidal (*Et*) and infratrochlear (*IT*) branches. As the infratrochlear nerve passes forwards over the medial smooth muscle (*MM*), it gives off the sympathetic supply to it (*nMM*) and then ends by dividing into sensory branches (*S*). The superior oblique (*SO*) (retracted laterally) and its trochlear cartilage (*TC*) are seen, as well as the superior rectus (*SR*).

Fig. 2. Contents of the floor of a right orbit, viewed from the lateral side. The eyeball has been removed, leaving the optic nerve (*II*) (including optic head), the nictitating membrane, including the cartilaginous portion (*C*), and the remaining structures have been rotated medially in order to reveal the inferior aspect of the inferior smooth muscle (*IM*). The trigeminal nerve (*V*) can be seen dividing into ophthalmic (1), maxillary (2) and mandibular (3) divisions. The maxillary division (2) first gives off the lacrimal nerve (*L*) (which redivides) and then the zygomatic nerve (*Z*) (which was cut short in this dissection). The nerve to the inferior smooth muscle (*nIM*) enters the inferior smooth muscle (*IM*) on its infero-lateral aspect. The infra-orbital nerve (*O*) can be seen running along the floor of the orbit. Scale in mm. squares.

Plate 2

Fig. 1. Right orbit viewed from above. Superior oblique (*SO*) retracted laterally to show ethmoidal nerve (*Et*) and infratrochlear nerve (*I*) which has been cut proximal to the level at which the sympathetic supply (*nMM*) to the medial smooth muscle (*MM*) is given off. Before entering the medial muscle (*MM*), the sympathetic nerve redivides. During the experiment the cartilage was bisected and the half (*Cm*) belonging to the medial muscle can be seen with a ligature passed through the free edge. Contractions of the upper (medial) half of the nictitating membrane elicited by stimulation of the cervical sympathetic, were abolished by cutting what was believed to be the infratrochlear nerve; the dissection shows that the infratrochlear nerve was cut. Scale in mm. squares.

Fig. 2. Same orbit as in Fig. 1, contents of the floor viewed from the lateral side. The eyeball (*E*) has been collapsed and the remaining structures including the lower (inferior) half of the cartilage (*Ci*) of the nictitating membrane, rotated medially in order to reveal the contents deep to the inferior smooth muscle (*IM*). The sympathetic nerve to the inferior muscle (*nIM*) has been cut approximately midway between its origin from the zygomatic nerve (*Z*) and the level at which it enters the inferior muscle (*IM*); there was no lacrimal nerve in this specimen. The infra-orbital nerve (*O*) can be seen travelling along the floor of the orbit. Contractions of the lower (inferior) half of the nictitating membrane elicited by stimulation of the cervical sympathetic were abolished by cutting what was believed to be the sympathetic nerve supplying the inferior muscle; the dissection shows that this nerve was cut.

THE ACCESSORY MENINGEAL ARTERY OF MAN*

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INTRODUCTION

The accessory meningeal artery usually receives only perfunctory mention in most textbooks of anatomy. In the average textbook account the artery at times is described as a branch of the middle meningeal artery; at other times as a branch of the maxillary artery. The account is continued by stating that the accessory meningeal enters the foramen ovale and supplies the semilunar ganglion and adjacent dura mater. Throughout the paper this version will be referred to as the 'stereotyped' or 'abbreviated' account.

During routine laboratory dissections, our attention was initially called to two cadavera in which the accessory meningeal arteries failed to conform to the stereotyped description in that much of their distribution went to extracranial structures. Perusal of accounts of the artery in texts and atlases revealed a diversity of descriptions, which, coupled with additional observations in the laboratory, led to the conclusion that the accessory meningeal artery should be re-investigated.

HISTORY

Numerous anatomical works have been consulted in tracing the record of the accessory meningeal artery. These range chronologically from mid-eighteenth century to contemporary, and include as many as could be obtained of the widely used and acknowledged classical texts and references. In the historical synopsis set down below an attempt has been made to include representative works of each generation. It has become evident to us that the treatment accorded the acc. mening. a. has, in general, degenerated or devolved with the passage of time rather than evolved into more perfect form. That is, some of the earlier anatomists have described the acc. mening. a. much more adequately than have their successors. (Michels (1960) has commented on an analogous situation with regard to the middle hepatic artery.)

In the historical survey we noted:

(1) that the acc. mening. a. is often described as an inconstant vessel;

(2) that many authors have failed to call attention to the variable origin of the artery;

(3) that many authors have overlooked the rather extensive extracranial distribution of the artery or have shifted inordinate emphasis over to the intracranial distribution.

The acc. mening. a. has been called by a multiplicity of terms; according to the Paris Revision of the Nomina Anatomica (1955) it is designated as the Ramus meningeus accessorius of the Arteria meningea media. In light of the findings of

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the present study, it seems that A. meningea accessoria is a less inappropriate name, the anglicized form of which will be followed throughout this paper. Further remarks on this topic are made in the discussion.

Other synonyms by which the acc. mening. a. has been known are set down below:

A. meningea parva, R. meningeus parvus, small meningeal artery;

A petite méningée, arteria parvae, lesser meningeal artery;

A. meningea media parva.

Parvidural branch of middle meningeal artery.

A. meningea media accessoria, accessory middle meningeal artery.

A. pterygoidea externa.

A. ptérygo-méningé, R. meningeae pterygoideus.

A. meningica accessoria.

The earliest account of the acc. mening. a. available to us is from the Icones of Haller (1745). According to Barclay (1812) Haller was the first anatomist to describe minutely the int. max. a. Haller says that the acc. mening. springs from the max. a. anterior to the mid. mening., but most often from the mid. mening. itself. He cites the earlier works of Winslow and Gunz who also noted the origin from max. a., and observes that the main stem of the acc. mening. enters the cranium via foramen ovale. A more comprehensive description is that of Sömmering (1792) who indicates that the acc. mening. stems from the int. max. a. and distributes to both pterygoid mm., to mm. of the auditory tube, to the third branch of the fifth nerve, and to dura mater. He did not treat the course and relationships as thoroughly as Haller.

Barclay in his description of the arteries of the human body briefly alludes to the acc. mening. a. thus: '...a Ramulus is observed running along the third branch of the fifth pair of nerves to the Dura Mater.' He refers to it as a branch of the int. max. a. According to Meckel (1832) the acc. mening, is an inconstant branch of the int. max., which often arises from mid. mening. or a pterygoid artery, that sends branches to pterygoid mm., mm. of soft palate, and sometimes into skull to dura mater near the sella turcica. Meckel observes that the small mening. a. sometimes replaces deficient extracranial collaterals of the mid. mening. a. Bell's Anatomy (1834), referring to the int. max. a., states only, 'a branch of it sometimes goes into the skull by that hole named foramen ovale...and goes to that part of the dura mater which covers the sides of the sella turcica.' The most comprehensive and accurate of the earlier presentations appears to be that of Bourgery (1835). This author says the acc. mening., frequently lacking, springs from either mid. mening. or int. max. trunk distal to the origin of the inf. dental a. He describes its ascent between the pterygoid mm., providing variable arterioles to both of these muscles and at the base of the skull the artery's division into two rami: the *decending ramus* which winds about the superior attachment of the med. pt. m. and is lost in the wall of the nasal fossa and the soft palate; the *ascending ramus* which penetrates into the cranium, distributes to the dura and trigeminal nerve, and there anastomoses with rami of the internal carotid and mid. mening. aa. Tiedemann's plates (Knox, 1835) of the arteries of the human body fail to illustrate the acc. mening. a.

Wistar's text (1843) indicates only that the acc. mening. arises from int. max., ascends to foramen ovale, and passes to dura after having supplied branches to external pterygoid and mm. of the palate. Quain's monograph (1844) on the arteries

discusses the acc. mening. only in a legend to a plate. He states the artery originates from mid. mening., giving off a small branch which ramifies 'over the large plexus of veins in this situation after having furnished a meningeal branch through the foramen ovale of the sphenoid bone...'. Quain's and Wilson's Atlas (1845) calls attention to a feature unmentioned in earlier works. This is the course of the acc. mening. in relation to the 'pterygoid plate and circumflexus palati'. The only distribution indicated is to dura. Cruveilhier's (1851) description of the acc. mening. does little more than paraphrase the excellent one of Bourgery; however, the name, A. ptérygo-méningé, applied by Cruveilhier to the artery is significant and will be mentioned later. Power's (1860) account of the artery adds nothing despite his extensive section on variation. Barkow's plates (1866) offer no references to the acc. mening. a.

The works of both Luschka (1867) and Henle (1876) provide satisfactory descriptions; both mention origin of the acc. mening. from mid. mening. or int. max. aa. and distribution to pterygoid mm. Luschka includes rami to the soft palate and mandibular nerve. Henle adds the auditory tube and descending palatine mm. Both authors indicate that the acc. mening. terminates within the cranium. The eighth edition of Quain's Elements (Sharpey, Thomson & Schäfer, 1876) merely presents an abbreviated version. Gegenbaur (1896) mentions the variable origin of the artery as well as rami to pterygoidei and palate. He furthermore correctly notes the relatively small size of the intracranial ramus. Spalteholz, in the first edition (1898) of his Handatlas, as well as later ones, departs from the usual accounts of the artery by describing *only* an extracranial distribution of the acc. mening. a. Testut (1900), Poirier & Charpy (1901), Gray's Anatomy (1901), Cunningham (1906), Sobotta & McMurrich (1911), Testut & Jacob (1914), and Corning (1914) write only abbreviated versions of the acc. mening. a. Only a few of these signify any distribution other than the familiar 'semilunar ganglion and adjacent dura'. The variable origin of the artery is referred to only rarely. Chiarugi (1924), Gérard (1921), Toldt (1928), and Piersol (1930) likewise provide only stereotyped accounts of the artery. Adachi's (1928) monograph on the arterial system of the Japanese has no textual description of the acc. mening. and only a single unlabelled figure to show its existence. Tandler's (1926) account of the artery is similar to those of Luschka and Henle.

Later editions of some of the long-in-print, standard texts and atlases already cited, as well as many of the newer works on gross anatomy, have been reviewed. Most show no noteworthy improvements in the treatment of the acc. mening. a. However, from this assemblage, attention should be directed to the texts of Benninghoff (1952) and Rauber-Kopsch (Kopsch 1955) who properly indicate the supply of the pterygoid mm., mm. of the palate, as well as the auditory tube, semilunar ganglion and dura. Pernkopf's textual description of the artery (1957) is the stereotyped one. Several of his plates (1952, 1957) accurately illustrate (but do not designate) some of the principal and consistent extracranial rami we have observed. Paturet (1958) neglects to mention many of the extracranial structures supplied by the acc. mening. but does give the most comprehensive description of supply to intracranial structures we have encountered.

Our review of the literature failed to reveal works dealing specifically with the acc. mening. a.

MATERIALS AND METHODS

This investigation is based upon the results of seventy-six dissections of cadavera most of which were also utilized by medical and dental students. Specimens in which the antero-medial region of the fossa was disturbed were arbitrarily excluded from consideration. The series represents bilateral dissections of thirty-five specimens and unilateral dissections of six specimens. The first thirty dissections were performed by the junior author, the remainder by the senior author. The dissections were accomplished with the aid of a binocular microscope, and were recorded by means of detailed, annotated drawings. Especial care was taken to trace all rami of the acc. mening. a. into muscle, bone, etc., as far as possible. A comparison of sexual, racial, or age differences was not undertaken.

OBSERVATIONS

General description

The following is a generalized description of the acc. mening. a. as seen in our dissections. (See section on variation for departures from this description.)

Origin and Course. The acc. mening. a. originates either from the mid. mening. a. or from the mandibular or pterygoid segments of the max. a. and assumes a course somewhat parallel to the obliquely disposed superior border of the med. pt. m. The acc. mening. a. courses supero-rostrad toward the angle formed by the postero-superior border of the lateral pterygoid lamina and the infra-temporal surface of the great wing of the sphenoid bone (pterygoid portion of Facies temporalis, P.R. of Nomina Anatomica.) Here the vessel gives off its major terminal rami which distribute to structures in this region, including the interior of the middle cranial fossa. (For details, see section on distribution and Fig. 1.)

Relationships. Whatever its origin, the acc. mening, a. is related laterally to the deep surface of the lat. pt. m. Usually the artery passes medial to the inferior alveolar and lingual nerves. Posteriorly it may have a slight medial relationship to the lev. v. pal. m., particularly when it is a branch of the superior segment of the mid. mening. a. Further anteriorly it has extensive medial relationship to the ten. v. pal. m. When the acc. mening. arises further rostrally from the pterygoid part of the max. a., it courses between the lat. pt. m. and the tens. v. pal. The artery usually does not course between the pterygoid muscles since it is situated above the superior border of the med. pt. m.

In taking the path described above, the acc. mening. travels in the fascial plane which separates the two pterygoid muscles, the pterygoid fascia. According to Hovelacque & Virenque (1913) and Paturet (1951), the fascia of the region is further distinguished as an internal interpterygoid aponeurosis or lamina and an external pterygo-tempero-maxillary lamina enclosing an interaponeurotic space. This space contains the acc. and mid. mening. aa., the posterior division of the mandibular nerve, the auriculo-temporal nerve, part of the pterygoid venous plexus, as well as the so-called 'vascular sheath' of areolar connective tissue (described by the above authors) which accompanies the ascending branches of the max. a. These fascial relationships are illustrated in Fig. 2.

The often-thickened, superior border of the pterygo-tempero-maxillary lamina is called the pterygoalar ligament (of Hyrtl) or, when ossified, the pterygoalar bar. The opening between ligament (or bar) and the great wing of the sphenoid forms the pterygoalar foramen (porus crotophitico-buccinatorius of Hyrtl). The superior border of the interpterygoid aponeurosis is most often developed into the rather definite pterygospinous ligament (of Civinini), occasionally ossified as the pterygospinous bar. The opening bridged below by the latter forms the pterygospinous foramen (of Civinini). See Priman & Etter (1959) for a discussion of these structures.

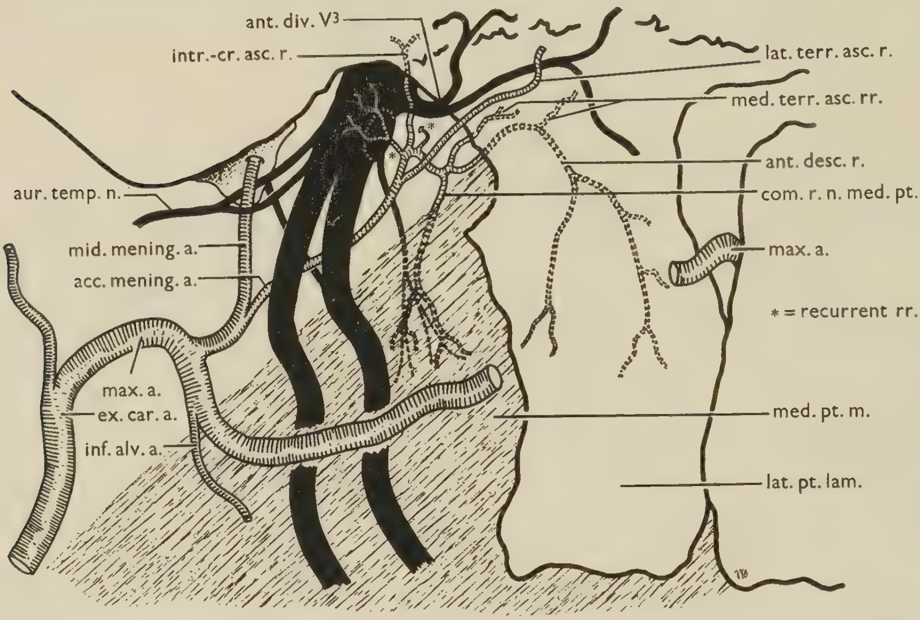

Fig. 1. Relationships and terminal distribution of the acc. mening. a. This is a composite based on the entire series of dissections, showing the most frequent occurrence and disposition of branches of the artery. (See text, section on terminal distribution.) ant. desc. r., anterior descending ramus; com. r.; n. med. pt., companion ramus of medial pterygoid nerve; intr.-cr. asc. r., intracranial ascending ramus; lat. terr. asc. r., lateral territory ascending ramus; med. terr. asc. rr., medial territory ascending rami.

The pterygospinous foramen is completed by a relatively delicate upward continuation of the interpterygoid aponeurosis called the 'cribriform fascia' by Hovelacque and Virenque. This fascia is pierced by the stem of the acc. mening. a. (and nerves to med. pteryg. and tens. v. pal. mm.) which loops mesad over the pterygospinous lig. to pass deep and leave the interaponeurotic space via pterygospinous foramen. A consistent ramus of the acc. mening, to the superior head of the lat. pt. m. leaves the space by swinging laterad and transversing the pterygoalar foramen in company with the deep temporal and buccal nn. (Fig. 2).

Distribution. The greatest part of the distribution of the acc. mening. a. supplies structures outside the cranial cavity. In the first part of its course it gives off variable collateral rami to both pterygoid mm., inferior alveolar and lingual nn.,

tens. v. pal. m., and connective tissue. On reaching the angle between great wing of sphenoid and lateral pterygoid lamina the stem of the acc. mening. breaks up into a spray of terminal rami. The latter supply the origin of med. pt. m.; superior head of lat. pt. m.; tens. v. pal. m.; root of mandibular nerve and otic ganglion; semilunar ganglion and adjacent dura; root of pterygoid processes and great wing of sphenoid bone. The structures listed above are arranged in descending order of importance to indicate the relative proportion of the distribution of the acc. mening. a. each receives.

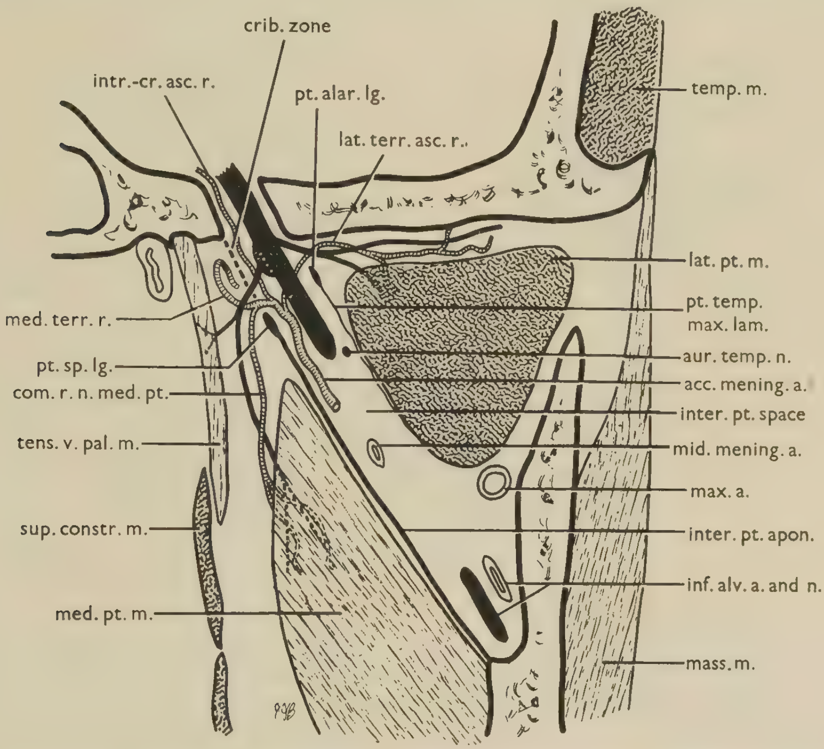

Fig. 2. Schema to demonstrate territories of distribution and fascial relationships of the acc. mening. a. (See text, section on distribution.) crib. zone, cribriform zone of interpterygoid aponeurosis; inter. pt. apon., interpterygoid aponeurosis; inter. pt. space., interpterygoid space; pt. alar. lg., pterygoalar ligament; pt. sp. lg., pterygospinous ligament; pt. temp. max. lam., pterygo-tempero-maxillary lamina.

Variation

Frequency of occurrence. Although many descriptive accounts of the acc. mening. a. indicate that it is a vessel of inconstant occurrence, no quantitative data in support of these statements have been found. Because of its inconsistent origin and its variable course within the pterygoid fascia and venous plexus, the acc. mening. a. is easily overlooked. The 'inconstancy' of the artery may well be attributable to the above circumstances. Our observations show that the acc. mening. a. is almost always present. It was demonstrated in seventy-three of seventy-six dissections or 96 % of our series.

Size. Although no measurements were made, the calibre of the usual acc. mening. a. appears to be about one-third to one-half that of its corresponding mid. mening. a. A few acc. mening. aa. were observed to be nearly as large as the mid. mening. of the same side. Cruveilhier notes one case wherein the acc. mening. equalled the mid. mening. in size.

Origin (Fig. 3). The acc. mening. a. arises with about equal frequency from the first or second parts of the max. a. (thirty-four cases) or from the mid. mening. a. (thirty-five cases). When the latter is the parent vessel, the acc. mening. usually originates along its proximal half. When the max. a. is the parent vessel, about

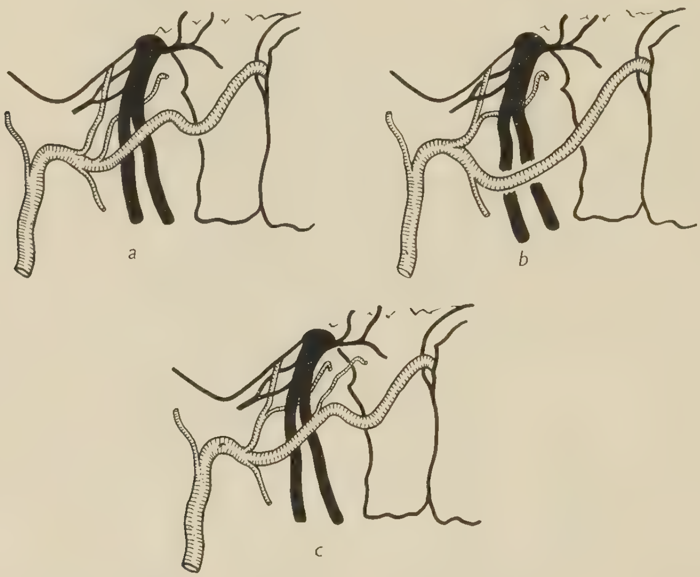

Fig. 3. Principal sites of origin of acc. mening. a. (*a*) origin from max. a., 34 of 73 sides; (*b*) origin from mid. mening. a., 35 of 73 sides; (*c*), 'dual' origin from both max. a. and mid. mening. a., 4 of 73 sides. (Compare with Fig. 1 for details; see text, section on origin.)

60 % of acc. mening. aa. arise from the segment posterior to the inf. alv. and ling. nn.; 40 % come off the segment of the max. a. rostral to the nerves.

In fifty-five of the seventy-three dissections the acc. mening. originates as a single stem. In the remaining group the artery is represented by more than one vessel. Often these multiple arteries originate from the parent vessel quite near to one another. In others their origins are relatively distant from one another. Since the multiple arteries correspond so closely in course and distribution to the single-stem arteries, they are judged to represent the acc. mening. and are treated as such. A larger proportion of acc. mening. aa. arising from the max. a. are multiple than are those from the mid. mening. From the max. a., 21 are single, 9 double, 2 triple, and 1 quadruple. From the mid. mening., 33 are single, and 2 double. In four dissections there occurs what we call 'dual origin', i.e. the acc. mening. is represented by two rami, one from the mid. mening. and another from the max. a. of the same side (see Fig. 3).

Symmetry of origin. Of the 35 specimens dissected bilaterally, 14 (or 40%) demonstrate an acc. mening. a. springing from the max. a. of one side and from the mid. mening. contralaterally. Of the remaining 21 bilaterally dissected specimens, the acc. mening. aa. of:

10 specimens arise from the max. aa. of both sides;

8 specimens arise from the mid. mening. aa. of both sides;

2 specimens are present on one side and lacking contralaterally;

1 specimen are dual—arise from both mid. mening. and max. aa. bilaterally.

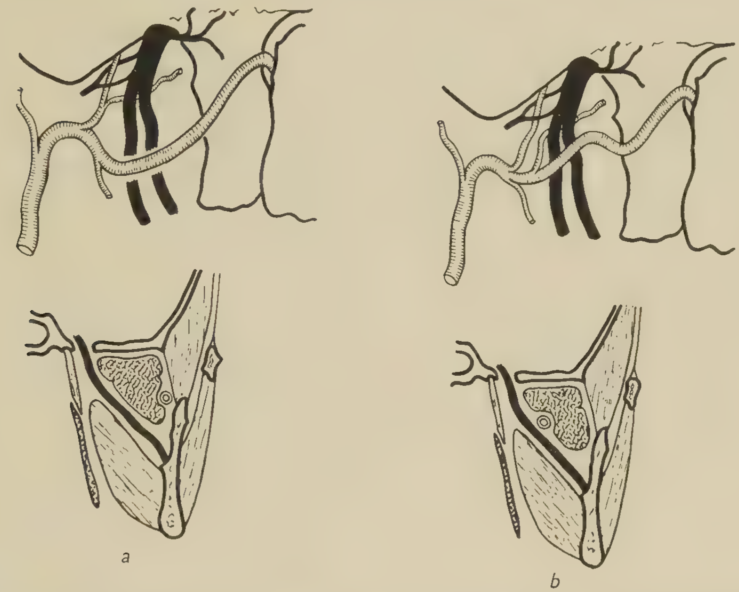

Fig. 4. Correlation or origin of acc. mening. a. with the disposition of the max. a. (54 observations). Lower figures show the disposition of the max. aa. to the lateral pterygoid mm. The upper figures show the usual site of origin of the acc. mening. a. in each type. (*b*) deep variety of max. a. Origin of acc. mening. directly from max. a. (*a*) superficial variety of max. a. Origin of acc. mening. from mid. mening. a.

Correlation of origin with disposition of max. a. It is well known that the max. a may cross the infratemporal fossa on a plane either medial or lateral to the lat. pteryg. m. When the max. a. is disposed medial to the muscle it is referred to as the deep or interpterygoid variety. When the artery occupies the lateral position it is known as the superficial or ante-pterygoid variety (Paturet, 1958). The relative frequencies of occurrence of these varieties have been studied by Lauber (1901) and Lurje (1947) among others.

A definite relationship has been found to exist between the situation of the max. a. and the origin of the acc. mening. a.:

When the max. a. is of the deep variety, the acc. mening. stems directly from the maxillary trunk; when the max. a. is superficial, the acc. mening. arises from the mid. mening. (see Fig. 4).

The disposition of the max. a. to the lat. pt. m. was recorded in fifty-four dissections.

In all but 8 of the 54, the above agreement or correlation held true. Statistical tests were applied to these data to determine their validity. Using the technique of 'significance of difference of proportions', a *P*-value of $> 0{\cdot}0002$ was obtained—highly significant statistically. It is therefore possible to predict with a high degree of assurance the parent stem of the acc. mening. a. only by knowing the variety of max. a. which a specimen possesses.

Relationship to mandibular nerve (Fig. 5). The relationship of the acc. mening. a. to the posterior division of the mandibular nerve varies both between individuals

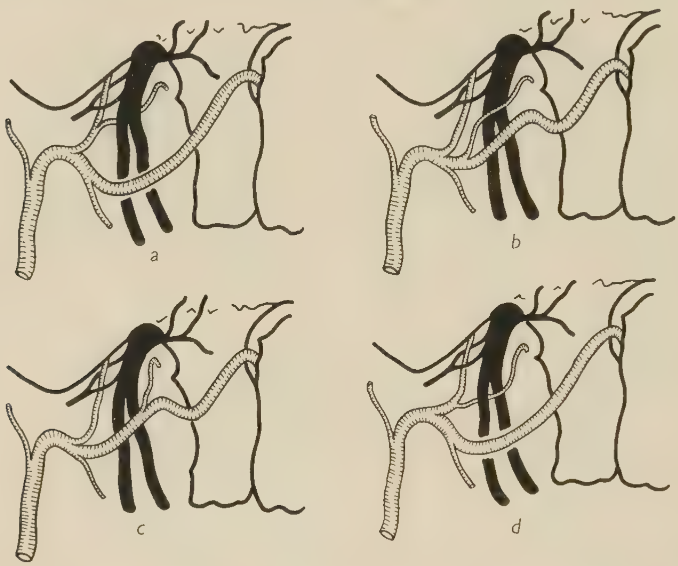

Fig. 5. Relationship of acc. mening. a. to mandibular nerve. (*a*) acc. mening. medial to nerve, 40 of 73 sides; (*b*) acc. mening. lateral to nerve, 14 of 73 sides; (*c*) acc. mening. rostral to nerve. 15 of 73 sides; (*d*) acc. mening. splits nerve, 4 of 73 sides.

and between sides of the same individual. The artery commonly (40 cases) passes on the medial aspect of the nerve (or of its two principal branches, the inf. alv. and ling.). Less often (14 cases) the acc. mening. courses lateral to the nerve(s). In four cases the artery splits the nerve in different ways: passing between and separating ling. from inf. alv. or actually piercing the substance of the mandibular nerve or one of its branches. In the fifteen remaining cases the acc. mening. originates from the max. a. rostral to the nerves and thus has no medio-lateral relationship to them. However, the artery often ascends in the fossa along the rostral border of the mandibular nerve. Not uncommon when the acc. mening. passes deep to the nerve is a direct relationship to the N. chorda tympani. The artery ascends, then loops rostrad over the nerve, lying clasped in the angle between chorda and its junction with the lingual n. (Fig. 1). At times the acc. mening. loops over the inferior root of the auriculotemporal n., dragging this root caudad.

Distribution

Classification of the branches of the acc. mening. a. is complicated by a notable amount of individual variation in the pattern of branching. In spite of the variation several consistently occurring branches are met with in practically every specimen. Furthermore, certain territories supplied by the acc. mening. a. can be delineated irrespective of the variation in course and branching of rami to these territories.

Four regions of distribution or territories are arbitrarily recognized. Fig. 2 will assist in visualizing these. The *lateral territory* comprises those structures superficial to the pterygo-tempero-maxillary lamina. The lateral pterygoid process itself forms the forward extent of this plane and is included in this territory. Also part of this territory is the infratemporal surface of the great wing of the sphenoid bone. The *medial territory* encompasses those structures deep to the interpterygoid aponeurosis. The *intracranial territory* consists of structures in the foramen ovale and in the middle cranial fossa. The *interpterygoid territory* consists of the connective tissue, veins, and nerves in the interval comprising the interaponeurotic space. Every acc. mening. a. in the series possesses extracranial branches. In no case was all of the distribution intracranial.

In each dissection the proportion of the distribution of the acc. mening. to each territory has been estimated. The estimate is simply a subjective comparison of the number and sizes of branches passing to each territory, expressed in percentage.

Medial territory. This territory receives the greatest share of the distribution of the acc. mening. a. in 61 of 73 cases. Throughout the series the average share to this territory is about 55 % (range 25–95). Medial territory rami are always present. The chief structure supplied in this region is the med. pt. m.; the tens. v. pal. m. ranks next in importance. Other medial territory structures which receive rami are: periosteum and bone of the medial pterygoid lamina, root of great wing of sphenoid, root of the pterygoid processes, anterior wall of the pterygoid fossa., lev. v. pal. m., and auditory tube (infrequently).

Lateral territory. This region received most of the distribution of the acc. mening. a. in 10 of 72 dissections. In seven instances no lateral territory rami were observed. The average share of the distribution to lateral territory is about 30 % (range 0–60). The lat. pt. m. (particularly its superior head) is the lateral territory recipient of most consequence. Other structures often supplied by rami of the acc. mening. are: periosteum and bone of the infratemporal surface of the great wing of sphenoid, lateral pterygoid lamina; the proximal parts of the anterior division of the mandibular nerve (temporal, buccal, masseteric nn.); connective tissue and pterygoid venous plexus.

Intracranial territory. In only 2 of 72 instances (3 %) does this territory receive the bulk of the distribution of the acc. mening. a. The average share to this territory is only about 10 % (range 0–40). An intracranial ramus to the part of the mandibular nerve lying in the foramen ovale or into middle cranial fossa occurs in 60 of 73 cases (82 %). An intracranial distribution is thus not of constant occurrence, nor are the intracranial rami very often sizeable.

Since the middle cranial fossae of most of the specimens of the series had been previously dissected, we have been able only to make a limited number of observa-

tions of the terminal distribution of intracranial rami. Many of the latter lose themselves in the substance of mandibular nerve root and surrounding dura in foramen ovale, and have only an insignificant extent in the cranial cavity. Sizeable intracranial rami (seen in the few specimens with intact middle fossae) distribute largely to dura; only a minor share of the terminals pass to the semilunar ganglion. Some twigs were seen supplying the intracranial segments of the ophthalmic and maxillary nerves. Intracranial rami of the acc. mening. do not always enter the cranium via the foramen ovale (see below).

Interpterygoid territory. The average share of distribution of acc. mening. a. to this territory is estimated at about 5 %. In all specimens minute, variable twigs arise from the acc. mening. in its course through the interaponeurotic space. These twigs supply the abundant adipose and areolar connective tissue here. Probably the most significant rami are those vasa nervorum to the inferior alveolar and lingual nerves, the undivided stem of mandibular nerve and otic ganglion. Not uncommon are rami which accompany the Chorda tympani.

Terminal distribution. Fig. 1 is a composite featuring the most consistently occurring branches of the acc. mening. a., depicting their most common origin and pattern of distribution.

The acc. mening. almost invariably ascends to a position anterior to the mandibular nerve, just inferior to the base of the skull, where it yields its terminal branches. For convenience of description these are classified as *ascending*, *descending*, and *recurrent* rami. It will be recalled that the acc. mening. attains the above position by coursing in the interaponeurotic space; within the space the recurrent branches, the intracranial branches, and one important lateral territory ramus arise from it (Fig. 2). The stem of the artery arches sharply mesad over (or rarely through) the pterygospinous ligament or bar, penetrates the cribriform fascia in order to leave the interaponeurotic space.

The artery here lies encompassed by the labyrinthine pterygoid venous plexus in a fascial cleft between the fascia of the lateral surface of the tens. v. pal. m. (fascia of Weber-Liel) and the deep surface of the interpterygoid aponeurosis (cribriform zone). This plane continues inferiorly between the tens. v. pal. and the med. pt. m. It is on this plane that the medial territory ascending and descending rami originate.

Descending rami. There are usually two prominent descending rami. A very constant one descends between the tens. v. pal. and the med. pt. m., takes up company with the med. pt. n., and with the latter penetrates the medial surface of the muscle. This companion ramus of the med. pt. n. (A. pterygoida medialis, Pernkopf, 1952) occurs in 66 of 73 sides (90 %), and often supplies the tens. v. pal. as well. At times it arises well forward under cover of the lateral pterygoid lamina, recurving markedly to join the nerve. In a few instances a collateral twig of this branch passes down to end in the sup. constr. m. of the pharynx.

The other descending branch is the apparent continuation of the trunk of the acc. mening. This anterior descending ramus arches rostrad in the roof of the pterygoid fossa above the uppermost fasciculi of origin of the med. pt. m. It then curves gradually inferad along the anterior wall of the pterygoid fossa. Generally it descends between the tens. v. pal. and the med. pt. m. and, at times, is seen to accompany

N. tensoris veli palatini. Sometimes the artery descends in the substance of the med. pt. m., and less frequently it travels between the inner surface of the lateral pterygoid lamina and the med. pt. m. At the top of its arch a nutrient twig to the roof of the pterygoid fossa is common.

In the lower part of its course, the anterior descending ramus distributes to the tens. v. pal., the origin of the med. pt. m., periosteum and bone of the medial pterygoid lamina, and anterior wall of the pterygoid fossa. Not infrequently this ramus bifurcates, one division going into the origin of the med. pt. m. The other division courses on a deeper level along the anterior, free border of the tens. v. pal., occasionally winding mesad around this border, providing twigs to the deep surface of the tensor, the auditory tube, and lower part of the lev. v. pal. In a few instances terminals of the anterior descending ramus pass to the postero-lateral wall of the nasal fossa or to the upper surface of the soft palate.

Ascending rami. Three ascending branches of the acc. mening. are usually present one each to lateral, medial, and intracranial territories. The *lateral territory ascending ramus* originates in the interaponeurotic space, turns laterad as it rises to leave the space (via pterygoalar opening), and enters the medial aspect of the superior head of the lat. pt. m. and the tissues between this head and the infratemporal surface of great wing of sphenoid. This branch supplies the muscle, periosteum and bone of the upper part of the lateral pterygoid lamina and the adjoining surface of great wing of sphenoid, the roots of temporal, buccal, and masseteric nerves, connective tissue, and pterygoid venous plexus. The lateral territory ascending ramus often springs from the more proximal extent of the acc. mening. a. In fact, when the acc. mening. is represented by plural arteries, this lateral territory ramus often springs independently from the mid. mening. or from the distal extent of the max. a. (deep variety) before the latter crosses the lateral pterygoid lamina (see Fig. 3*c*).

A *medial territory ascending ramus*, appearing very frequently, is a nutrient artery to the sphenoid bone, which enters the root of the pterygoid processes or the root of the great wing of sphenoid by a large single foramen or smaller multiple foramina located just anterior to foramen ovale and lateral to scaphoid fossa. This nutrient ramus may originate from a vessel common to it and the above lateral territory ramus. Or it may arise from the anterior descending ramus. Unless traced out fully the nutrient ramus may easily be confused with an intracranial ramus; however, its course is antero-superior, not vertical. The *intracranial ascending ramus* generally courses toward the anterior angle of the foramen ovale and enters the foramen anterior or medial to the mandibular nerve.

Not all intracranial rami utilize the foramen ovale. The sphenoidal emissary foramen (of Vesalius) conducts an intracranial ramus of the acc. mening. a. in 16 of 73 sides (22 %). The foramen is situated 2–3 mm. medial to the anterior end of the foramen ovale and also transmits an emissary vein. Most references which mention the sphenoidal emissary foramen indicate that it contains only the vein. Weber (1842) and Gegenbaur (1896, p. 246) point out that the acc. mening. a. can pass into the cranial cavity through its own special opening, although neither author applied a name to the opening. LeDouble (1903) mentions an accessory spheno-spinous foramen which sometimes transmits the small mening. a. His account,

however, is confusing, and it is not clear whether the latter foramen is synonymous with the sphenoidal emissary foramen.

Small intracranial rami usually dissipate themselves in the segment of the mandibular nerve (or its epineurium) which lies in the foramen ovale. In certain cases a small ramus enters a bony foramen corresponding in position to a sphenoidal emissary foramen, but it becomes lost in bone and cannot be located on the cerebral surface of the great wing. Larger intracranial rami on attaining the interior of the cranium pierce the dura at variable levels, passing into the semilunar (Meckel's) cave or into the cavernous sinus. In only a few instances were we able to trace the terminals of sizeable intracranial rami. In these the ramus usually bifurcates into a small twig which runs posterad to supply dura on the side of the sella turcica and the semilunar ganglion. A larger anterior twig runs on the medial aspect of the roots of the maxillary and ophthalmic nerves supplying vasa nervorum to them as well as twigs to neighbouring dura. Corresponding arteries directly from the internal carotid have been observed in several specimens which lack intracranial rami of the acc. mening.

Recurrent rami form a variable group which pass generally posterior-superior. Most of these remain in the interaponeurotic space and distribute to the undivided stem of the mandibular nerve and to the otic ganglion. Others on a deeper level turn medially into the lateral surface of tens. v. pal. m. In one case a rather large medial twig was seen to pierce the origin of the tensor and pass to the auditory tube in the region of the junction of its cartilaginous and osseous segments. A recurrent ramus in another instance passes posterad between the deep surface of mandibular nerve and tensor, finally reaching the lateral surface of the lev. v. pal. m.

DISCUSSION

The historical review of the acc. mening. a. in this paper points out that an erroneous, abbreviated description of the artery has become well established and perpetuated in anatomical literature. This stereotyped version of the acc. mening., for the most part, omits reference to an extracranial distribution and stresses only that part of the artery which enters the skull through the foramen ovale. It has been difficult to comprehend why this version has not been previously questioned, since from time to time for the past 200 years rather good descriptions of the artery have appeared. In part our findings direct attention to existing, essentially correct accounts of the acc. mening. a., but many of our observations add new information.

The frequency of occurrence of the acc. mening. a. (96 %) is definitely much higher than we had originally anticipated. On the basis of our series of dissections, the acc. mening. should be considered as a ubiquitous vessel.

Even the better versions of the acc. mening. a. fail to place proper emphasis on the primarily extracranial distribution of the artery. We have estimated that on the average only approximately 10 % of the blood carried by the artery supplies structures within the cranial cavity. Furthermore, an intracranial ramus of the acc. mening. does not always exist. Nor when one is present does it always enter the skull by way of the foramen ovale. In more than 20 % of cases it traverses the sphenoidal emissary foramen.

Blood supply of semilunar ganglion. The acc. mening. a. is reputed to be an important vessel of supply to the semilunar ganglion and adjacent dura. The question arises: what is the blood supply of the ganglion in the absence of an intracranial ramus? A imited number of dissections have been made on the available specimens with this in mind. We have seen direct branches of the intrasinus segment of the internal carotid pass to the ganglion when intracranial rami of the acc. mening. are lacking. These branches are the arteriae receptaculi. Further investigation along these lines is needed.

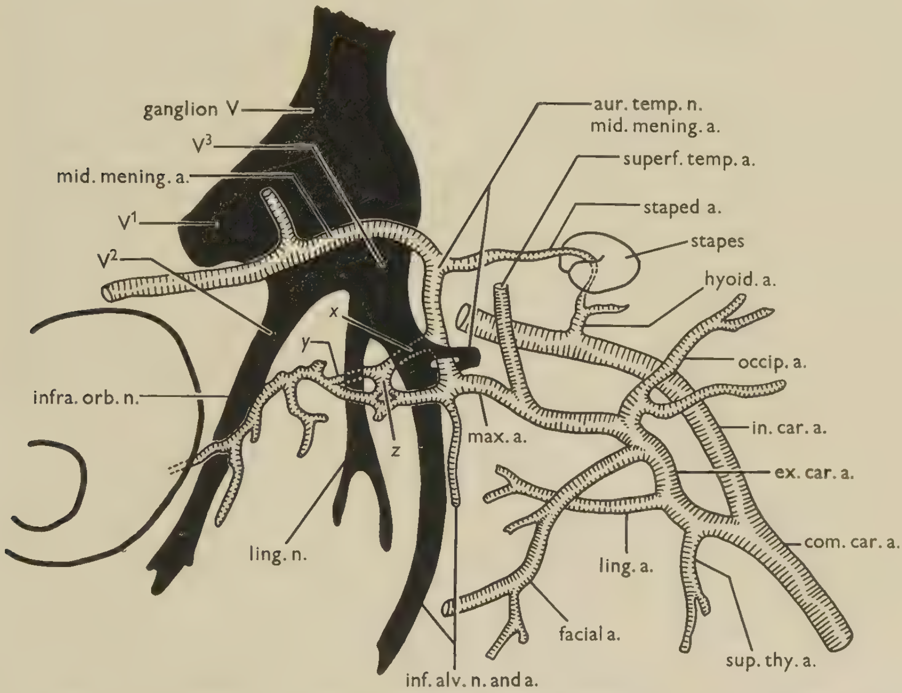

Fig. 6. Development of the acc. mening. a. 24 mm. human embryo. x, y, and z are channels comprising the medial loop of the primitive max. a. These channels probably persist in various fashions to produce the definitive acc. mening. aa. (compare with Figs. 3. and 5). Adapted from Padget (1948), fig. 8, p. 223, through the courtesy of the Carnegie Institution of Washington.

Vasa nervorum and nutrient supply to sphenoid bone. To our knowledge neither the vasa nervorum to the root of the mandibular nerve and otic ganglion nor the nutrient vessels to certain parts of the sphenoid bone have been previously ascribed to the acc. mening. a. (supply to the third branch of the fifth nerve was noted by Sömmering and Luschka.) Examination of the norma basalis of practically any macerated skull will demonstrate numerous small foramina on the infratemporal surface of the great wing of the sphenoid and its root, as well as on both pterygoid processes and their root. A rather large, constant nutrient foramen is situated in the roof of the pterygoid fossa. Certain of these foramina conduct nutrient arterial twigs from the acc. mening. into these parts, while others transmit veins which communicate with the pterygoid venous plexus. Paturet mentions only nutrient

twigs from the acc. mening. a. to the internal surface of the great wing of sphenoid and to the wall of the sphenoid sinus.

Branches of the acc. mening. to the auditory tube, lev. v. pal. m., nasal fossa, and soft palate proper, although described variously by some authors as regular branches of the acc. mening., have been rarely observed by us.

Development of accessory meningeal artery. Apparently the acc. mening. a. is derived from part of the embryonic primary medial limb of the arterial loop formed by the max. a. around the developing mandibular nerve. Fig. 6 is modified from Padget's (1948) work on the development of the cranial arteries in the human embryo. This figure depicts the medial limb, connected behind the nerve by a channel (x) to the mid. mening. a. and to the continuation of the stem of the max. a. in front of the nerve by a channel (y). A communicating channel (z) connects the medial and lateral limbs and intervenes between inferior alveolar and lingual nerves. Comparison of this figure with Figs. 3 and 5 shows that all of the three indicated channels correspond well with common sites of origin of the adult acc. mening. a. Furthermore, their relationships to the mandibular nerve closely resemble the adult relationships of the artery to the nerve. Thus there is reasonable evidence on which to presume that the definitive acc. mening. a. represents persistent portions of the medial limb of the primitive maxillary artery.

The origin of the acc. mening. a. has been seen to be correlated with the development of the max. a. The acc. mening. springs from the mid. mening. when the max. a. is the superficial variety. It springs from the max. a. itself when the latter is the deep variety.

Proposed terminology. Since the parent artery of the acc. mening. is the max. a. as often as the mid. mening., and since the main area of distribution of the artery is to the superior pterygoid region, we submit that versions of the terms acc. mening. or small mening. are not suitably descriptive. Reference is made to the table of synonomy in the introductory part of this paper. Both Haller and Cruveilhier applied the name pterygomeningeal for the acc. mening. a. Indeed, the latter author called for usage of pterygomeningeal. From the standpoint of descriptive aptness, as well as chronological priority, we suggest that the term. *A. pterygomeningeus*, is most appropriate for the artery.

SUMMARY

The common, stereotyped description of the accessory meningeal artery indicates only that it is inconstant, that it may arise from the middle meningeal artery or the maxillary artery and that it traverses the foramen ovale to supply the semilunar ganglion and adjacent dura. Only rarely is an extracranial distribution of the accessory meningeal mentioned.

Our investigation which is based on the results of seventy-six dissections shows that:

1. The accessory meningeal artery is practically always present (96 % frequency of occurrence).

2. The accessory meningeal artery originates with about equal frequency from either middle meningeal artery or the maxillary artery. A significant correlation

is found to exist between the disposition of the maxillary artery (deep or superficial to the lateral pterygoid muscle) and the origin of the accessory meningeal artery. The accessory meningeal artery arises directly from the deep variety of maxillary artery, but arises from the middle meningeal artery when the parent stem of the latter is the superficial variety of maxillary artery. The accessory meningeal artery is sometimes represented by plural vessels.

3. The distribution of the accessory meningeal artery is primarily extracranial. An intracranial ramus, when present, usually represents only a small fraction of the total distribution of the accessory meningeal. The accessory meningeal artery distributes principally to the medial pterygoid muscle, superior head of the lateral pterygoid muscle, tensor veli palatini muscle, parts of the sphenoid bone, middle cranial fossa, and the root of the mandibular nerve and otic ganglion.

4. The accessory meningeal artery apparently develops from persistent portions of the primary medial loop of the embryonic maxillary artery. Detailed accounts of the course, relationships, distribution, and variation of the accessory meningeal artery are included in the paper.

In terms of chronological priority and descriptive aptness, the name, *A. pterygomeningeus*, is considered more appropriate than accessory meningeal artery.

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THE CONTRIBUTION OF MIGRATORY EPITHELIUM AND THE RELATION OF WOUND ENLARGEMENT TO HEALING

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INTRODUCTION

The closure and repair of full thickness skin wounds of the trunk of most experimental animals is due mainly to contraction, a forced inward movement of the skin surrounding the wound in response to tensile forces generated within the wound, and to a lesser extent to the migratory activity of the epithelium of the wound margins (Spain & Loeb, 1916; Carrel & Hartmann, 1916). Although the process of contraction has been studied in considerable detail both with regard to its quantitative aspects (Billingham & Reynolds, 1952; Billingham & Medawar, 1955; Billingham & Russell, 1956*a*; Brenk, 1956) and to the factors causing it (Abercrombie, Flint & James, 1954; Grillo, Watts & Gross, 1958*a*, *b*; Cuthbertson, 1959; Abercrombie, James & Newcombe, 1960) there seems to be inadequate information on the contribution of the migratory epithelium to the repair of this type of wound. Carrel & Hartmann (1916) concluded that there was considerable variation in the area covered by epithelial growth from the edges, and that expansion of this area occurred subsequent to the complete epithelialization of the wound. This latter observation was confirmed by Abercrombie & James (1957) in their detailed and careful study in rats. On the other hand, Billingham & Medawar (1955) stated that 'migratory epithelium, granulation tissue and the product of its fibrous transformation are regarded as temporary organs of repair which disappear when their work is complete'. By means of a histological study similar to that used in investigating the healing of defects in the immobile skin of the ear of the rabbit (Joseph & Townsend, 1960), it seemed likely that additional and possibly more accurate qualitative and quantitative information could be obtained regarding the epithelium which spread from the edges of a wound in mobile skin, especially during healing.

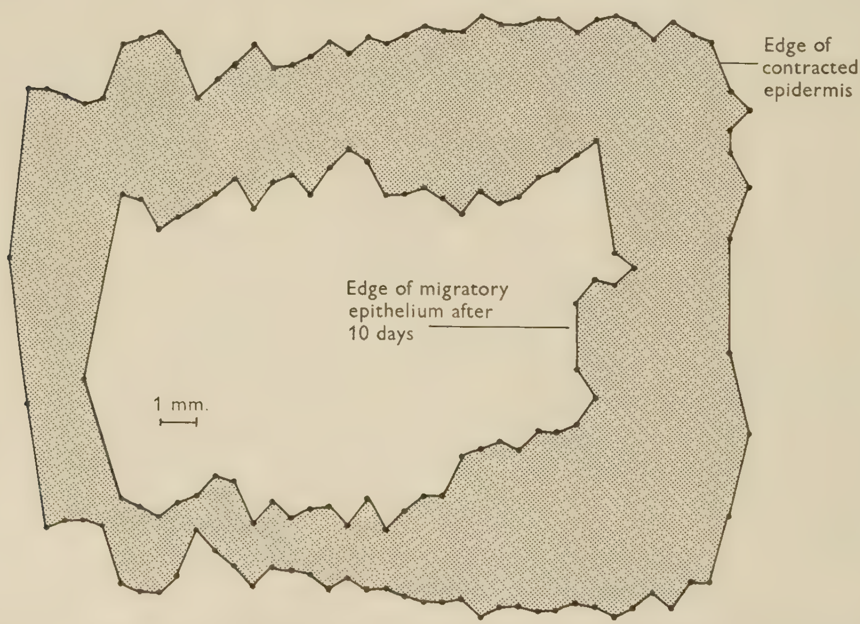
Owing to the elasticity of the skin the size of the wound after the removal of a standard area of skin varies considerably, and it was thought that there might be some relationship between the extent to which a wound enlarged and its subsequent rate of closure.

MATERIAL AND METHOD

Adult rabbits of mixed stock weighing between 2 and 3 kg. were used. All operations were carried out under Nembutal anaesthesia, supplemented by ethyl chloride as required. The hair was closely shaved on the dorsilateral thoracic wall with an electric clipper and the skin was cleaned with a solution of 1 % cetrimide. An area, 2×2 cm., was marked on the skin with ink and removed down to the panniculus carnosus. On removal of the skin, the wounds usually enlarged to some extent (Pl. 1, fig. 1). The wound was then dressed with penicillin tulle gras, gauze and

plaster of Paris, as described by Billingham & Medawar (1951). After 5 days for the 5-day specimens and after 10 days for all others, the dressings were removed and the wounds were allowed to heal without further dressings. Photographs were taken and observations recorded at intervals of 5–10 days. (The hair was clipped if it had regrown.) Four or five specimens after 5, 10, 15, 20, 30 and 100 days were obtained and these consisted of the whole thickness of the skin and the panniculus carnosus. In order to limit gross contraction of the specimen, the skin surrounding the wound, before being cut, was impaled on to a circular disc, 5 cm. in diameter, with eighteen cutting needles fixed on its periphery. The specimen was kept on this disc while being fixed in Bouin's fixative and was removed only before embedding.

Each specimen was then cut at 10μ and sections at intervals of 500μ were mounted and stained with haematoxylin and eosin. Many intervening serial sections were mounted and stained with Van Gieson's stain and Lillie's modification of Masson's trichrome stain for connective tissues, Best's carmine technique for glycogen, and periodic acid Schiff technique for mucopolysaccharides, Verhoeff's elastic tissue stain and Gomori's modification of the Bielschowsky–Maresch method for reticulin.



Text-fig. 1. Chart of 10-day migratory epithelium measured on sections at intervals of 500μ. Area in centre is still denuded and shaded area is covered by migratory epithelium.

Microscopically the migratory epithelium could be easily distinguished from the old epidermis except in the 100-day specimens and its horizontal extent was measured on each section at intervals of 500μ. These measurements, enlarged 10 times, were then plotted on graph paper and from these graphs the total amount of new epithelium was calculated by counting squares. This also enabled the remaining denuded area to be measured (Text-fig. 1). The original denuded area was measured on the photographs and the area of the wound covered by contraction was obtained

by subtracting the sum of the areas covered by migratory epithelium and still denuded from the original area. Since the specimen shrank during preparation both the area covered by migratory epithelium and the remaining denuded area (if any) were smaller than they actually were on the chest of the rabbit before biopsy. This method of measuring the amount of epithelial growth was used because it appeared to be more accurate than measuring by direct vision or photography, as the growing edge was often covered by a scab and even without a scab was not always clearly discernible macroscopically.

RESULTS

General. When the dressings were removed the wound showed some bleeding. After 5 days there was very little change in the wound macroscopically. A layer of granulation tissue covered the panniculus to a variable extent, sometimes completely, and was especially marked at the sides of the denuded area. The original sharp edges of the wound were rounded probably due to epithelial migration. After 10 days the wound was reduced in size, mainly by contraction, and the amount of migratory epithelium had increased. After 15 days there was a marked reduction in the size of the denuded area. This was largely due to contraction which had taken place in the manner described by Billingham & Russell (1956*a*)—the mid-points of the opposite sides of the wound approached each other, so that the edges of the wound were curved. At this stage, there was obvious epithelial growth from the edges of the wound and there was a scab over the unhealed area extending over the edges of the migratory epithelium (Pl. 1, fig. 2). After 20 days the wound showed only a very small central unhealed area and by 30 days the wound had completely healed (Pl. 1, fig. 3). In the 100-day rabbits, after 50 days, three out of five showed a cruciform growth of hair where the contracted edges of the wound had met (Pl. 1, fig. 4). This was sometimes striking because the surrounding hair had not grown so markedly.

Histology. In the healing specimens the junctions of the old and new epithelium could be clearly distinguished because of the increased thickness of the latter which consisted of about fifteen layers of cells. Nearer the centre of the wound the migratory epithelium thinned to a single layer of cells (Pl. 1, fig. 5). In a completely healed specimen after 30 days the new epithelium remained thicker than the adjacent old epidermis. The new epithelium could be divided into the usual layers, stratum germinativum, etc., although the cells in each layer appeared larger than in normal epidermis. During healing, there was a marked increase in the number of mitotic figures seen in the stratum germinativum and stratum spinosum of the new epithelium behind the advancing edge. The amount of glycogen in the stratum spinosum increased, especially in the 5-, 10- and 15-day specimens. By 30 days the glycogen content was normal. The new epidermis up to 30 days did not show any hair follicles.

In the 5- and 10-day specimens, granulation tissue was seen below the new epithelium and extended over the remaining denuded area. As the wound edges approached each other the granulation tissue shrank and was still found mostly beneath the migratory epithelium and in the denuded area. Meanwhile certain changes had occurred in this tissue. By 15 days it contained many fibres, mainly reticular, which were thin and ran in all directions. By 20 days some collagen fibres

were present, but there were no elastic fibres. By 30 days the collagen fibres had increased in quantity and there were a few elastic fibres often running perpendicular to the surface. The collagen fibres were arranged in bundles running generally parallel to the surface. During the early stages in wound healing there was an increase in the mucopolysaccharides in the granulating tissue. This, however, decreased as the collagen fibres began to form and mature. The tissue which had developed from the granulation tissue and was deep to the new epidermis could be easily distinguished from the normal dermis because its fibres did not run in a haphazard direction and appeared to be longer, more continuous and arranged parallel to the surface. This was probably due to the fact that the tissues were continually being pulled by the same tensile forces as produced contraction of the surrounding skin and collagen fibres tend to be laid down parallel to lines of stress.

The 100-day specimens merit special description. The new epidermis was much thinner than that seen earlier on (after 30 days) and numerous hair follicles, smaller than normal, were seen. These changes made it difficult to measure the area covered by the new epidermis but the underlying new connective tissue remained quite distinctive due to its increased cellularity, density and parallel arrangement (Pl. 1, figs. 6, 7). This tissue could be followed from the deeper parts of the section where it lay under normal skin, towards the surface where it was seen under the new epidermis. The interruption in the normal superficial dermis could be easily seen so that the site of the new epidermis could be confirmed.

Quantitative studies. Table 1 gives the original denuded areas (measured on the photographs), the areas covered and those still denuded (measured on the charts) and the areas covered due to contraction (obtained by subtracting the sum of the areas covered and those still denuded from the original denuded areas). It is clear that there is variation in the size of the denuded areas due to retraction of the skin edges after removal of a standard area of skin. The mean area is $6{\cdot}84 \pm 0{\cdot}23$ cm.^{2}. These figures are in close agreement with those of Abercrombie *et al.* (1960).

Table 2 gives the means and standard errors of the means of the areas covered by migratory epithelium after various intervals of time. By an analysis of variance (Table 3), it can be shown that the areas covered by the new epithelium after the intervals studied differ significantly ($F = 3{\cdot}96, f_1 = 4, f_2 = 18, 0{\cdot}025 > P > 0{\cdot}01$). This analysis however does not locate these differences. This may be done by a sequential method of testing (Snedecor, 1956; $s_{\bar{}} = 0{\cdot}124$, $a = 6$, $f = 22$) which shows that the mean area covered by new epithelium is significantly greater at 10 and 15 days than at 5, 30 and 100 days, and that the mean area at 20 days is significantly greater than at 5 and 30 days ($P < 0{\cdot}05$). None of the other differences are significant at the 5% level. One may therefore conclude that some of the migratory epithelium acts as a temporary cover and then disappears.

The scatter-diagram (Text-fig. 2) presents the original areas plotted against the areas covered by contraction 5, 10, 15 and 20 days following the standard wound. The data for the 30- and 100-day specimens have been excluded because at some time between 20 and 30 days all defects irrespective of their original area have been completely covered mainly by contraction (cf. the first and fourth columns for 30 days in Table 1). It can be seen that the area covered by contraction appears to vary with time, and that within each time-group the area covered by contraction

Table 1. *Original denuded areas, areas covered by migratory epithelium remaining denuded areas and areas covered by contraction (in cm.²)*

Period of healing (days)	Original denuded area	Area covered by migratory epithelium	Remaining denuded area	Area covered by contraction
5	8·12	0·48	3·93	3·71
	5·50	0·12	2·48	2·90
	5·52	0·34	3·59	1·59
	5·76	0·76	3·21	1·79
	4·20	0·80	2·92	0·48
10	6·16	0·51	2·05	3·60
	8·32	2·21	1·04	5·07
	6·84	1·68	1·01	4·15
	5·75	1·59	1·57	2·59
15	9·00	0·67	0·35	7·98
	6·28	2·49	0·50	3·29
	6·72	1·50	0·01	5·21
	7·50	1·06	0·11	6·33
	5·28	0·71	0·23	4·34
20	5·50	1·76	0·03	3·71
	7·84	1·12	0·01	6·71
	7·84	0·96	0·03	6·85
	6·25	0·46	0·02	5·77
30	5·06	0·35	—	4·71
	4·56	0·65	—	3·91
	5·52	0·52	—	5·00
	7·74	0·21	—	7·53
	8·16	0·44	—	7·72
100	5·75	0·69	—	5·06
	7·13	0·58	—	6·55
	6·98	0·61	—	6·37
	6·60	0·49	—	6·11
	5·60	0·68	—	4·92

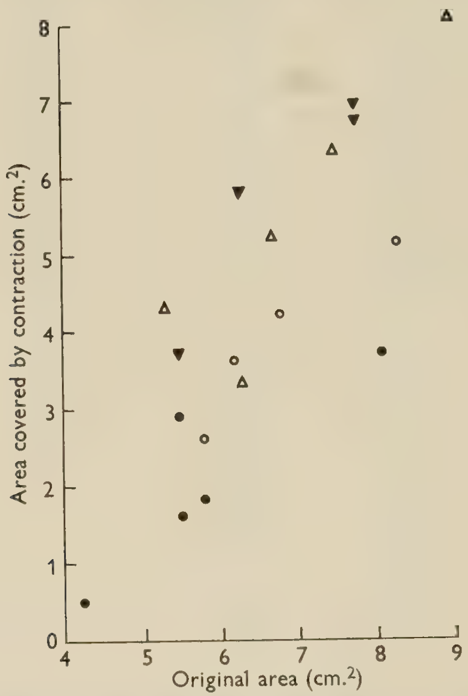
Table 2. *Means and standard errors (in cm.²) of areas covered by migratory epithelium after different periods of healing*

Period of healing (days)	Means and standard errors
5	0·50 ± 0·13
10	1·50 ± 0·36
15	1·29 ± 0·34
20	1·08 ± 0·27
30	0·43 ± 0·08
100	0·61 ± 0·03

Table 3. *Analysis of variance for areas covered by migratory epithelium*

Source of variation	Sums of squares	Degrees of freedom	Variance estimate
Time	4·57	5	0·91
Residual	5·11	22	0·23
Total	9·68	27	—

also apparently has a positive correlation with the original area. An analysis of variance (Table 4) confirms this. The effects of time ($F = 25{\cdot}84$, $f_1 = 3$, $f_2 = 10$, $P < 0{\cdot}005$) and original area ($F = 11{\cdot}52$, $f_1 = 4$, $f_2 = 10$, $P < 0{\cdot}005$) are both highly significant. It may therefore be concluded that the wound which originally expanded most after the standard procedure contracted most rapidly.



Text-fig. 2. Scatter-diagram showing original denuded area plotted against area covered by contraction after 5 (●), 10 (○), 15 (△) and 20 (▼) days.

Table 4. *Analysis of variance for areas covered by contraction*

Source of variation	Sums of squares	Degrees of freedom	Variance estimate
Time	39·94	3	13·31
Regression	23·71	4	5·93
Residual	5·15	10	0·52
Total	68·80	17	—

DISCUSSION

From these investigations it appears that it is only partially correct to describe the epithelium which migrates from the edge of a wound in the mobile skin of the rabbit as a temporary organ of repair. Some of the epithelium remains as a definitive part of the final healed state and contributes about 10 % to the coverage of the defect produced by the removal of an area 2 × 2 cm. in size. The amount of migratory epithelium reaches a maximum between 10 and 20 days. Unlike the findings of

Abercrombie & James (1957) in rats, there is no evidence that this area subsequently enlarges. This agrees with the observations of Billingham & Reynolds (1952) and Billingham & Russell (1956*a*) who noted that there was little or no expansion of the linear scar following the almost complete contraction of flank wounds in rabbits.

This new epidermis becomes much more like normal epidermis than that seen over large scars in that it develops hairs and is of average thickness. The appearance of new hairs confirms the observations of Breedis (1954) and Billingham & Russell (1956*b*). On the other hand, the underlying organized granulation tissue remains markedly different from the normal superficial dermis (Pl. 1, fig. 7).

There have been observations on the relation of the size of a wound to the rate of contraction. Young, Fisher & Young (1941) concluded that large wounds contract at a greater rate than smaller, but Billingham & Russell (1956*a*) suggested that the rate of contraction remained remarkably constant for wounds of very different size. It is obvious that in all these experiments the size of the defect in the skin on the trunk of a rabbit depends on (*a*) the amount of skin removed, and (*b*) the extent to which the edges of the wound retract. If the amount of skin removed is the same in each experiment, the final size of the defect will depend on the elasticity of the skin. It has been shown in the experiments reported here that the greater the retraction, the more rapid is the subsequent contraction. This is due to the tensile forces within the wound or at the edges of the wound (Grillo *et al.* 1958*b*). Thus the larger the wound, the more rapid is the contraction if the difference in size is due to a difference in the amount of retraction. This would explain to some extent the observations of Billingham & Russell (1956*a*) that there is a progressive decrease with age in the rate of contraction since in general there is a progressive loss of elasticity in skin with age.

SUMMARY

1. A standard area of skin 2×2 cm was removed from the chest in rabbits and dressings were applied for 5 or 10 days. Healing and healed specimens were obtained after 5, 10, 15, 20, 30 and 100 days.

2. By measurements on sections the areas covered by epithelial migration and those still denuded were determined.

3. There was a significantly greater amount of migratory epithelium after 10 and 15 days than after 5, 30 and 100 days. It is concluded that to some extent the migrating epithelium acts as a temporary organ of repair. Some of it however (about 10 % of the area denuded) is permanent.

4. It was also found that the greater the enlargement of the initial standard wound due to retraction, the faster was the rate of subsequent contraction. It is suggested that the more elastic the skin, the more rapid is the contraction.

5. Histologically after 100 days the migratory epithelium formed new epidermis which was as thin as the old and contained hairs, but the organized granulation tissue which remained was denser and more cellular than normal dermis and markedly different from it.

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EXPLANATION OF PLATE

Fig. 1. Wound on side of chest after removal of 2×2 cm. full thickness skin. Note the enlargement of the wound (each division equals 1 mm.).

Fig. 2. Healing of standard wound after 15 days showing the scab covering the edge of the migratory epithelium.

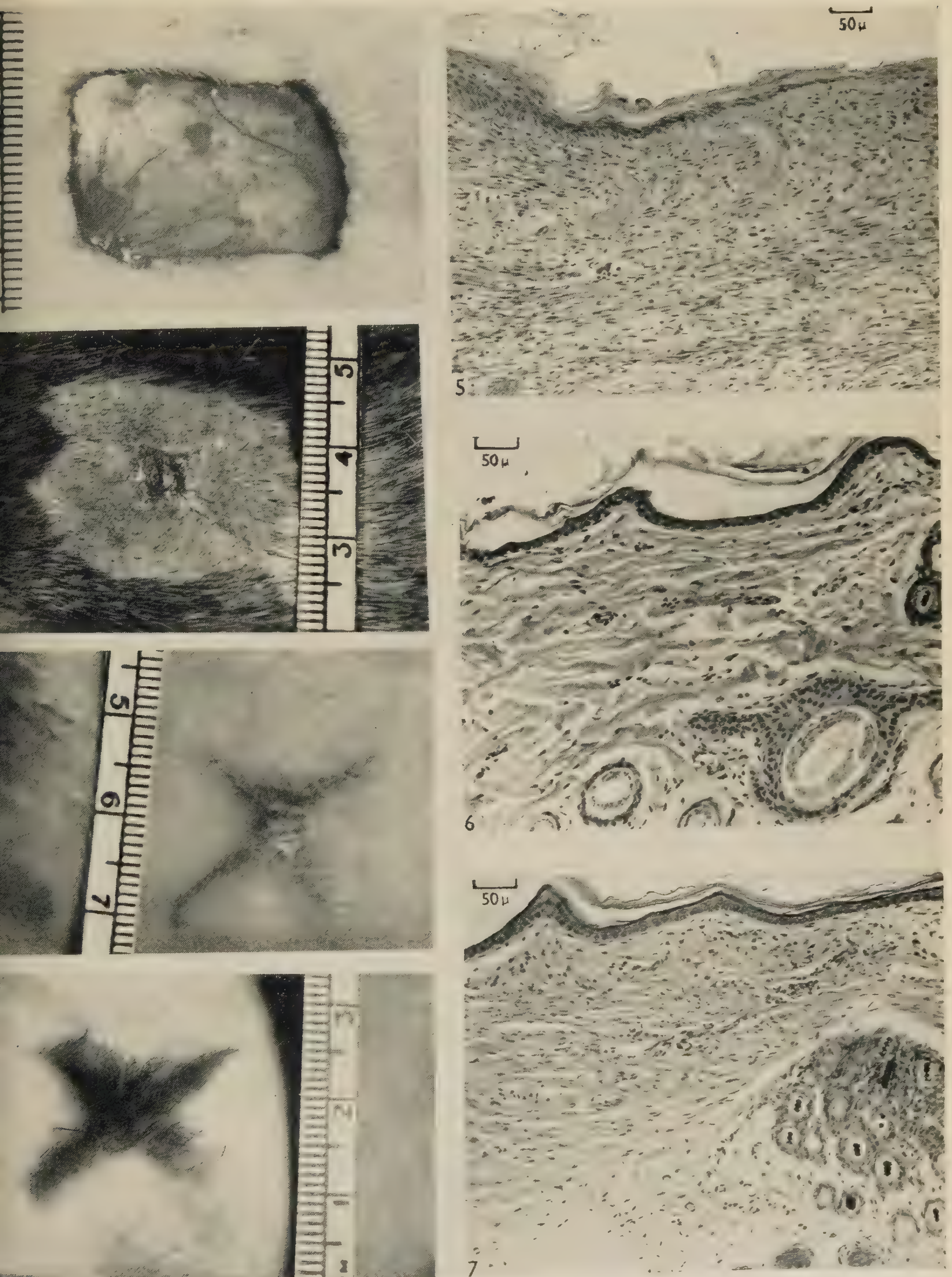
Fig. 3. Healing of standard wound after 30 days, showing the form of the final scar.

Fig. 4. Healing of standard wound after 50 days, showing hairs growing from scar.

Fig. 5. Section of healing skin after 15 days, showing migratory epithelium and underlying granulation tissue (H. & E.).

Fig. 6. Section of normal skin next to scar, showing usual arrangement of fibres in dermis (H. & E.).

Fig. 7. Section of scar part of healed skin after 100 days, showing thin epidermis with hair follicles, and dense, cellular connective tissue (H. & E.).



JOSEPH AND TOWNSEND—CONTRIBUTION OF MIGRATORY EPITHELIUM

(*Facing p.* 410)

EFFECT OF COMMERCIAL PARATHYROID EXTRACT ON EMBRYONIC AVIAN BONE *IN VITRO*

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INTRODUCTION

Gaillard (1955*a*,*b*; 1957; 1958; 1959; 1960) has worked extensively on the effects of living parathyroid gland tissue, cell-free fluid in which parathyroid tissue had been grown *in vitro*, and commercial parathyroid extract upon mouse parietal bone explanted *in vitro*. He observed (1960) 'an induction and/or stimulation of a process of lacunar bone resorption in the presence of many large multinuclear osteoclasts'; also, that 'the experimental environment was capable of creating conditions favouring the formation, survival, motility and specific functioning of osteoclasts'. These statements referred to explants known to contain osteoclasts at the start of the experiment; if osteoclasts were not present, they did not form *de novo*. In later experiments (1960), an increase in bone resorption and in the numbers of osteoclasts present occurred in the explanted mouse radius under the influence of commercial parathyroid extract ('PTE').

On the other hand, according to Kroon (1959) the primary effect of PTE, when injected into rodents, is upon the osteoblast. Within 3 days this cell dedifferentiates, proliferates, and lays down argyrophil fibres.

The experiments described below were carried out on embryonic avian bone with a technique permitting explants much larger than Gaillard's. They reacted towards PTE in a rather different manner, details of which are given below.

MATERIALS AND METHODS

Explants were prepared from the frontal bones of 14-day fowl embryos. The anterior portion was excised; the resulting piece, pyramidal in shape, measured roughly 4 mm. in length and tapered gradually from its anterior pointed end to give a cross-sectional diameter of approximately 1·5 mm. posteriorly. Such pieces were explanted whole on plasma clots in watch-glasses 5 cm. in diameter.

The clot was composed of 1 vol. of fowl plasma, 1 vol. of fowl embryo extract, and 1 vol. of Hanks' saline containing 10% horse serum. 'Parathormone' (Eli Lilley and Co.) was added to the latter solution just before use, as described by Gaillard (1957), to give final concentrations ranging from 0 to 12 i.u. per ml. of nutrient medium. The watch-glasses were placed upon perforated trays in flat glass vessels. After their lids, bearing a thickness of moistened gauze, had been applied, a stream of either air or of 5% CO_2 in O_2 was passed through for some minutes; the vessels were then finally sealed and incubated. They were regassed daily. The explants were transferred to fresh medium after 4 days and incubation continued in the same atmosphere as before.

After various time intervals the explants were fixed, sectioned and examined histologically. Samples prepared as for explantation were also fixed and examined prior to incubation.

RESULTS

A. *Effects of air and of* 5 % CO_2 *in* O_2

These experiments were carried out on control cultures, i.e. without the addition of PTE to the medium. There was a striking difference in the results. In an atmosphere of air (Pl. 1, fig. 1) bone formation had ceased after 4 days; active osteoblasts had practically disappeared, and osteoclasts had undergone obvious degeneration. On the other hand, with 5 % CO_2 in O_2 the condition of the cultures seemed excellent; the histological findings are described in more detail below. This atmosphere was therefore used for the remainder of the experiments.

B. *Control cultures*

(1) 2–4 *days* in vitro (32 *explants*)

In the absence of PTE the rather large explants had been maintained in surprisingly healthy condition (Pl. 1, figs. 2, 3). In some places well-preserved and active-looking osteoblasts, polyhedral in shape and with basophil cytoplasm, were associated with the formation of new bone. In others, osteoclasts of normal appearance had apparently continued to erode (Pl. 1, figs. 2, 3). The connective tissue cells of the intertrabecular spaces seemed unaffected by life *in vitro*. Compared with the pieces fixed before incubation, the normal processes of bone modelling seemed to have progressed more or less uninterruptedly.

(2) 4–8 *days* in vitro (16 *explants*)

Osteogenesis gradually declined with time. Osteoblasts (Pl. 1, fig. 4) had lost their basophilia and polyhedral shape and become flatter and inactive in appearance. Osteoclasts, on the other hand, seemed to have been affected much less and were of normal appearance. Intertrabecular connective tissue cells seemed normal and there were no signs of tissue necrosis even in the depths of the explants.

C. *Experimental cultures*

Various combinations of PTE dose level with time were studied. Since the best results with control cultures had been obtained with an incubation period of 4 days most of the tests with PTE were terminated at this time. However, a small number were maintained *in vitro* for 7 days. The concentrations studied were 3, 6 and 10 i.u. of 'Parathormone' per ml. of medium. After 4 days incubation, results were as follows.

(*a*) 3 *i.u.* (8 *explants*)

Osteogenesis had been arrested. Osteoblasts had lost their basophilia and polyhedral shape, and were assuming a more flattened spindle shape. They had also proliferated noticeably. Osteoclasts seemed unaffected.

(*b*) 6 *i.u.* (18 *explants*)

Dedifferentiation and proliferation of osteoblasts had progressed further. The intertrabecular spaces contained masses of spindle cells (Pl. 2, figs, 5, 6). Silver staining for reticular fibrils (Gomori's technique) showed that a deposit of fine argyrophil fibrils had developed around these cells (Pl. 2, figs. 7, 8). Osteoclasts seemed unaffected.

(c) 12 *i.u.* (18 *explants*)

Proliferation and dedifferentiation of osteoblasts was more marked still. The endosteal spaces were filled with these cells and the feltwork of argyrophil fibrils was even denser. Osteoclasts seemed unaffected.

(*d*) 6 *i.u. for* 7 *days* (8 *explants*)

No significant differences occurred during the additional 3 days of incubation. There seemed to have been some overall reduction in the cell population within the intertrabecular spaces; the argyrophil fibrils had thickened and collagen fibres had made an appearance. Osteoclasts seemed unaffected.

DISCUSSION

The primary effect of PTE was evidently upon the osteoblasts which dedifferentiated and proliferated. In doing so, they laid down a feltwork of argyrophil fibrils. This sequence which was seen consistently in all the experimental explants seems to mirror very closely the series of changes described by Kroon (1959) in rodent bone. During the first 3 days after injection of PTE Kroon noted that osteoblasts changed into 'long fusiform connective tissue cells' and formed argyrophil fibres. Somewhat analogous changes were also reported by Gaillard (1960) in the explanted mouse radius; he observed a transformation of osteoblasts and loose connective tissue cells into dense masses of spindle cells. However, these events do not seem to have occurred to a noticeable extent in his parietal bone explants. It is possible that on account of their relatively small volume they contained an insufficient number of responsive cells to survive explantation and life *in vitro*.

In contrast to Gaillard's findings, in the present experiments the osteoclasts on subjective examination did not seem to be more plentiful or more active-looking in the experimental than in the control cultures. This rather fundamental and interesting conflict may be explained in several ways.

First, the impression that osteoclasts were not affected in the present experiments may have been mistaken, and in reality osteoclastic activity was increased, but for various reasons this was not recognized. As mentioned above, numerous osteoclasts were present in the samples on explantation. The problem then is to show in an objective way that there were more in the experimental than in the control explants at the end of the incubation period. Gaillard, too, must have faced objective difficulties of the same kind. Theoretically, osteoclast counts could have been carried out on serial sections, but the large volume and number of specimens ruled this out as a practical possibility. It has to be recognized therefore that the apparent indifference of osteoclasts to PTE in the present experiments is a subjective finding

which lacks objective proof. A further complication is that in all probability some new osteoclasts would have been generated anyway, as incubation proceeded, as part of the normal expression of the maturation of the explants; Barnicot (1947) has shown how rapidly the osteoclast population rises in the skulls of neonatal mice with time.

A second explanation might be that the incubation period was too short. In Gaillard's parietal explants 4 days elapsed before osteoclasts became prominent; the avian explants were larger than those from the mouse parietal, and it might be that permeation of PTE from the medium to the interior was slower. Against this is the fact that Gaillard reported changes in his radius explants, probably of about the same bulk as the avian explants, within 2 days, and also there are the negative findings in the present experiments after 7 days *in vitro*.

A third possibility, which perhaps seems the likeliest, is that avian and rodent bone *in vitro* differ in their response to PTE. This is strengthened by the fact that bone from these species is known to react differently *in vitro* towards vitamin A (Fell & Mellanby, 1952).

Goldhaber (1958, 1960) has recently shown by means of motion picture studies that mouse osteoclasts *in vitro* are very much more active in an atmosphere of 5% CO_2 in O_2 than in air. No real comparisons can be drawn between this finding and the results described above. The latter, however, indicate that avian osteoclasts soon perish in air, but survive in the gas mixture, and to this extent support Goldhaber's work.

Finally, it is interesting that osteoclasts survived *in vitro* for as long as 7 days. Previous work (Hancox, 1946, 1956) suggested a much shorter lifespan; osteoclasts wandering out into the plasma clot from explants of bone were moribund after 48 hr. It is not possible to say at present why the cells survived longer inside explants. Whether this is due to conditions of oxygenation or some factor residing in the bone itself remains to be seen. However, this finding is more in line with the survival time of between 4 and 9 days suggested by Arnold & Gee (1957).

Thanks are due to Miss Sylvia Warner for technical assistance in this work.

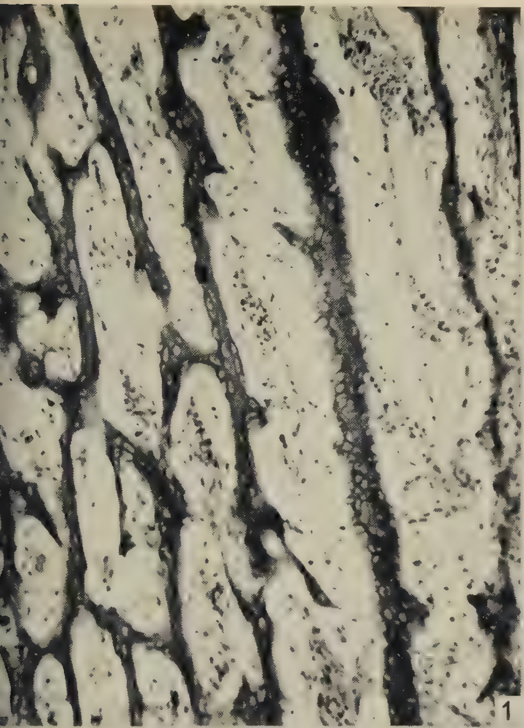
SUMMARY

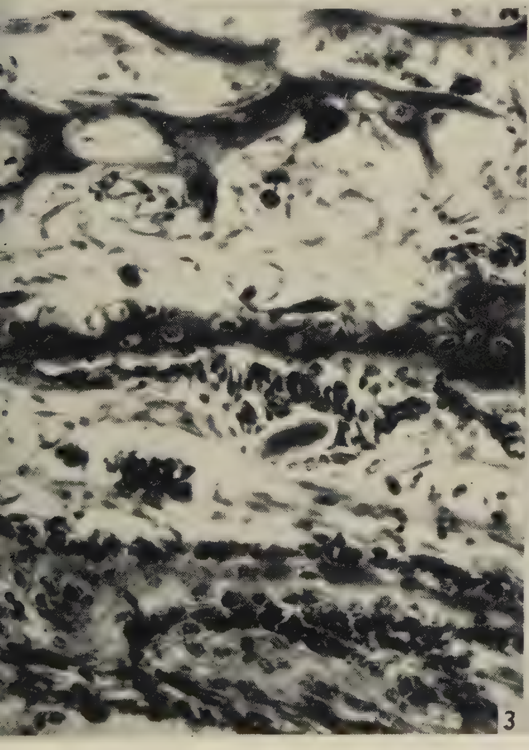
1. Pieces of skull bone from embryonic fowls were explanted on plasma clots in watch-glasses. An atmosphere of 5% CO_2 gave better results than air.

2. The normal modelling processes of deposition by osteoblasts and absorption by osteoclasts continued for about 4 days. Thereafter, osteogenesis gradually ceased whilst osteoclasts apparently continued unchanged.

3. Commercial parathyroid extract was added in concentrations of 3, 6 and 12 i.u. per ml. of medium and the effect studied histologically after 4 and 7 days. Compared with control cultures, the osteoblasts rapidly dedifferentiated, proliferated, and laid down fine argyrophil fibres. The osteoclast population was, apparently, unaffected.

4. These findings are discussed in relation to other reports of similar experiments. It is suggested that avian and rodent bone differ in their *in vitro* reactions to parathyroid extract.





HANCOX—EMBRYONIC AVIAN BONE *IN VITRO*

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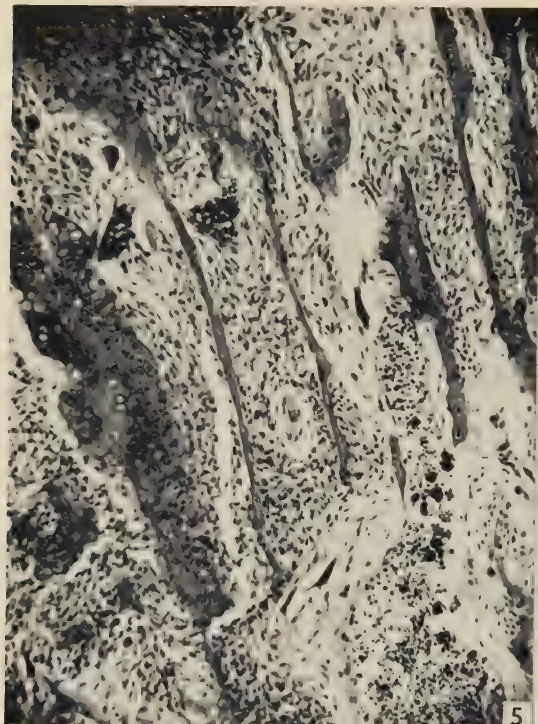

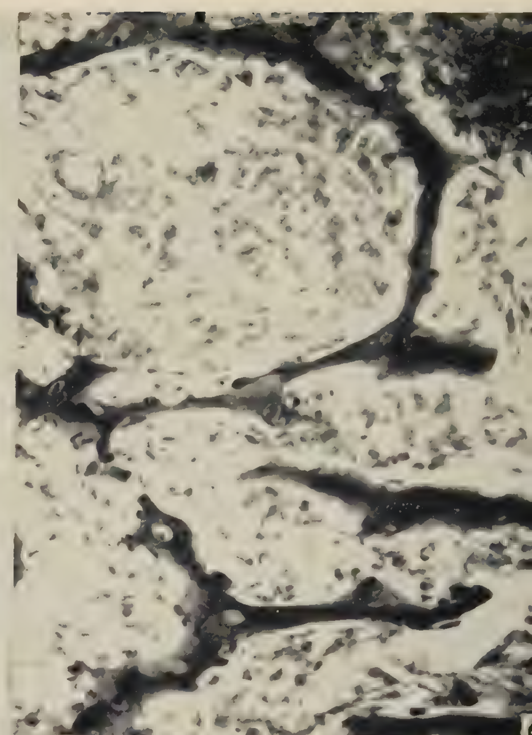

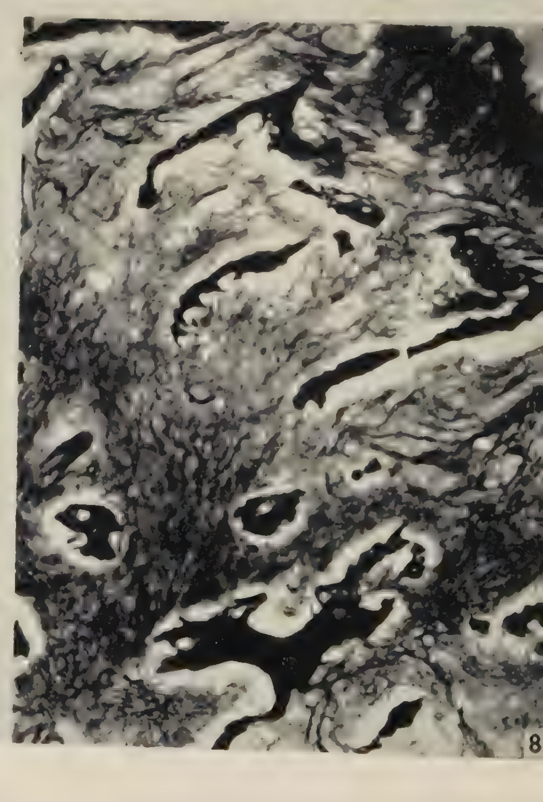

HANCOX—Embryonic avian bone *in vitro*

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. A portion of an explant after 4 days *in vitro* in an atmosphere of air. Osteoblasts have disappeared; osteogenesis has ceased completely. Scattered spindle cells are seen between the bone trabeculae. Blood vessels contain disintegrating cells. Compare Fig. 2. ×150.

Fig. 2. A portion of a control explant after 4 days *in vitro* in an atmosphere of 5 % CO_2 in O_2. Approximately one-third only of the sectional area is shown. Towards the right hand the bone trabeculae are lined by prominent osteoblasts; towards the left the osteoblasts are flatter and inactive. Some osteoclasts can be distinguished. Intertrabecular spaces contain sparse connective tissue cells and remains of blood vessels. ×150.

Fig. 3. Higher power view of portion of a control explant after 4 days *in vitro*. The edge of the explant appears at the bottom where active osteogenesis is apparent. Active osteoblasts line the lower surface of the trabeculum lying horizontally in the centre, and osteoclasts are visible above. ×320.

Fig. 4. Portion of a control explant after 7 days *in vitro*. Compared with Fig. 2, active osteoblasts have disappeared and osteogenesis has halted. Intertrabecular spaces contain healthy connective tissue cells. ×150.

PLATE 2

Fig. 5. Portion of an explant after 4 days in medium containing 6 i.u. 'Parathormone' per ml. The intertrabecular spaces contain masses of proliferating spindle cells derived from de-differentiated osteoblasts. Osteogenesis has ceased. ×150.

Fig. 6. Higher power view of culture similar to preceding. Many spindle cells lie between bone trabeculae. ×320.

Fig. 7. Portion of a control explant after 4 days *in vitro*. Gomori's silver method for reticular fibres. A fairly loose network of comparatively thick fibres lies between the trabeculae. ×320.

Fig. 8. Portion of a culture after 4 days in medium containing 6 i.u. parathormone per ml. Staining as in preceding. Compared with Fig. 7, there is a very marked increase in the intertrabecular argyrophil fibrils.

OBSERVATIONS ON THE INTRINSIC INNERVATION OF THE RECTUM AND ANAL CANAL

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Although many investigators have contributed to our knowledge of the composition, structure and function of the enteric nerve plexuses, scant attention has been paid to their termination in the anal canal, or to the junctional zone where somatic and autonomic innervation meet or overlap. It was therefore clear that this problem deserved close study.

MATERIALS AND METHODS

Forty-seven animals, of both sexes, were used for this investigation. These included seven monkeys, six cats, six rabbits, sixteen guinea-pigs and twelve rats. In addition an attempt was made to correlate the findings in experimental animals with those in man by an examination of silver preparations of tissue obtained from five human subjects at operation. The operations were performed for carcinoma of the rectum, but in no case was the anorectal junction involved in the disease process.

Routine histological studies of the anal canal were carried out using longitudinal and transverse paraffin sections of 10μ thickness stained with haematoxylin and eosin. A modified Bielschowsky technique (Rintoul, 1960) was used to stain frozen sections and 'stripped' preparations of the intestinal canal. This method was controlled using Gomori's (1937) reticulum stain.

In addition, supravital methylene-blue techniques were used. The methylene-blue solution found best was similar to that described by Mitchell (1953), and was maintained at a temperature of 37° C. The intestine was removed from the animal (which had been freshly killed by an overdose of 'Nembutal'), distended with methylene blue and submerged in a similar solution, staining being controlled by microscopy. The material was fixed in ice-cold saturated ammonium molybdate for 3 hr., dehydrated with dioxane, cleared in xylol and mounted in 'Dee-P-X'.

Gold staining techniques (Cole, 1946) and Champy's (1913) osmic acid technique were also employed.

Method of orientation of tangential sections of the anal canal

Following a method described by Dixon (1958), the piece of tissue to be sectioned was placed horizontally on the stage of the freezing microtome and frozen. Small holes were then made with a dental drill through the section to mark the anal end, the rectal end, the pectinate line and the white line. Prior to this, these landmarks had been verified by using stained longitudinal sections. Thus any future horizontal section could be mounted correctly and its various parts identified in relation to the epithelium. Serial sections were then cut, silver-stained and mounted. By a careful study of these sections it was possible to determine the way in which the enteric plexuses terminated.

OBSERVATIONS

The general histological division of the anal canal into three main areas is associated with pronounced differences in their pattern of innervation. The rectum and upper part of the anal canal are provided with simple tubular glands which terminate at the pectinate line, where the epithelium becomes stratified squamous in nature. This uncornified epithelium is continuous at the white line (Hilton, 1863) with true cornified skin containing sweat glands and hair follicles. The longitudinal muscle of the rectum is joined, in many animals, at the anorectal junction by fibres from the levatores ani and ends by dividing into fibrous strands which are attached to the skin at the white line, below the internal sphincter, to the lower part of the anal canal and to the perianal region (Wilde, 1948–9).

The nerve plexuses

The lower part of the rectum possesses a profuse nerve plexus situated in the areolar tissue surrounding the organ from which nerve fibres pass inwards to join both the myenteric and perivascular plexuses. This perirectal plexus contains no ganglia.

The myenteric plexus (Auerbach, 1864) is situated between the two muscle layers and the pattern of its network varies in different animals. For example, it is rectangular in the rat (Pl. 1, fig. 1) and hexagonal in the guinea-pig (Rintoul, 1957, 1958). Slender fibre bundles branching from the main or primary plexus and occasionally containing nerve cells, unite to form a secondary plexus (Auerbach, 1864; Stöhr, 1932) lying within the meshes of the primary one. This secondary plexus is continuous with an even finer tertiary web just visible in Pl. 1, fig. 1, and shown more clearly in Pl. 1, fig. 2. The tertiary net is composed of nerve fibres overlaid by the beaded processes of the autonomic interstitial cells (Cajal, 1911), about whose nature and function there is still much controversy; different authorities have regarded them as connective tissue elements or as microglial or nervous in nature (Dogiel, 1895, 1899; Kuntz, 1922; Hill, 1927; Boeke, 1940). The tertiary net is directly continuous with the plexus muscularis profundus (Pl. 1, fig. 3) which lies inside each muscle coat and consists of nerve fibres running parallel to the muscle strands along with interstitial cells and their processes.

The ganglia which are placed at the interstices of the primary network contain many nerve cells of types I and II (Dogiel, 1899). Pl. 1, fig. 4, illustrates three of the type I cells in the lower part of the rectum of the guinea-pig. Pericellular networks could be found around cells of both types.

In the anal canal the myenteric plexus can be traced in the above form as far distally as the pecten where the ganglia are more sparsely distributed. Pl. 2, fig. 5, shows a ganglion in the myenteric plexus lying in the pecten of the cat; it contains mainly unipolar cells but one bipolar neuron is clearly visible. This plexus ended at the level of the white line, sending many nerve fibre bundles below and through the internal sphincter to join the submucous plexus of the pecten.

The submucous plexus (Meissner, 1857) in the rectum is considered by some authorities (Stöhr, 1932; Okhubo, 1936) to consist of two closely knit plexuses—the plexus entericus internus (Henle) being that portion in close apposition to the

circular muscle layer and the plexus of Meissner being that portion situated nearer the lumen. These plexuses were found in the lower rectum to contain neurons of both types (cf. Kuntz, 1922; Stöhr, 1932), but the cells were of a smaller size than in the myenteric plexus. Nerve fibres from the submucous plexus penetrate the muscularis mucosae and form a fine periglandular plexus. Pl. 2, fig. 6, illustrates a nerve fibre bundle emerging from the internal sphincter of the cat. It joins the submucous plexus which, in turn, is seen to be continuous with a fine periglandular plexus surrounding the bases of the simple tubular glands of the anal canal.

Type I ganglion cells can be traced in the anal submucous plexus only as far distal as the pectinate line, none being found below this level. From the submucous plexus in the pecten some unmyelinated nerves join perivascular plexuses and others run singly in the connective tissue, while thickly myelinated nerves are connected with sensory corpuscles. Some fibres pass superficially, lose their myelin sheaths and join a subepithelial plexus which, in turn, gives rise to freely terminating intercellular, intraepithelial fibres. The patches of stratified epithelium occasionally found above the pectinate line are supplied in a similar manner.

The complexity of the specialized sensory endings varies in different species, all gradations from simple unencapsulated coils to more highly organized branched encapsulated endings being found. The nerve ending (Pl. 2, fig. 7) was obtained from a section of the pecten of a human. Many encapsulated sensory endings are apparently joined by an unmyelinated fibre in addition to the main nerve fibre. In one case two sensory corpuscles were observed to be connected by an unmyelinated nerve. Pacinian corpuscles (Pl. 2, fig. 8) were found lying deeply in the submucosa and also in the interval between the internal sphincter and the subcutaneous portion of the external sphincter.

Below the level of the white line the submucous plexus is continued as a subcutaneous plexus with no associated sensory corpuscles. Nerve plexuses around small blood vessels, and also around the bases of hair follicles, are present in abundance.

Innervation of the external sphincter

The external sphincter has a very profuse somatic innervation. In gold chloride preparations as many as sixty-five motor end plates were visible in one low power field in the guinea-pig. The shape of the end plate varies considerably and this variation cannot be accounted for solely by sectioning in different planes. The motor nerves which are thick and myelinated branch several times before reaching the muscle fibres, each branch passing to about the middle of the fibre. There is often a slight thickening in the myelin sheath before its termination and the neurilemma apparently fuses with the sarcolemma; the nerve fibre then divides into a number of fibrils which appear to end hypolemmally. At the motor end plate the muscle sarcoplasm is thickened and somewhat granular (Pl. 2, fig. 9).

In silver preparations (Pl. 2, fig. 10) several nuclei may be observed surrounding the hypolemmal terminations. No epilemmal motor end plates were observed, nor were accessory endings to the main plate noticed.

Between the muscle fibres there is a profuse network of unmyelinated axons. Apparent free endings of these upon muscle fibres were observed, but were considered to be artefacts produced by the tearing of fine nerves.

DISCUSSION

Very few reports can be found in the literature concerning the intrinsic innervation of the anal canal. Hilton (1863) described pudendal nerve filaments passing between the internal and external sphincters exactly underneath the white line and proceeding both towards the skin of the anus and towards the lower part of the rectal mucous membrane. Pilliet (1892) examined a portion of the anal canal of the human and commented on the presence of Pacinian corpuscles lying between the smooth and striated sphincters and suggested that, by acting as pressure receptors, they played a role in defaecation reflexes. Similar corpuscles in the anal canal were described by Winckler (1957) and by Japanese workers. Stroud (1896) described the innervation of the pecten in detail. He found an epidermal plexus of nerve cells of 10–15 μ in length and 6–7 μ width chiefly between the basal epidermal nerve cells but also more superficially and deeper in the dermis. There were frequent anastomoses of their dendrites. It was impossible to confirm his findings and his diagram illustrating these 'nerve cells' bears a striking resemblance to the dendritic cells described by Billingham (1948) in the guinea-pig. Stroud further described large multipolar dermal ganglion cells from 21–109 μ in length; these 'cells' gave rise to large processes and also to numerous small, hairlike processes of unknown destination. His drawing of a 'ganglion cell' contains three globular bodies and it is difficult to understand precisely what he was describing. It might be a true ganglion which contains nerve cells (the globular structures might then be nuclei or neurons), or he could have stained the intersection of fibre tracts in a plexus containing both myelinated and unmyelinated fibres. Both these would produce the same appearance, but the latter suggestion seems more probable.

Anal papillae developed from the tip of the pectineal dentations peculiar to man were also described by Stroud. These reputedly contained fine nerve fibres and ganglion cells. The papillae were further described by Abel (1932), and Ottaviani (1940) claimed that the anal columns were innervated by nerve fibres of the somatic type.

The few previous attempts made to describe the enteric plexuses in the anal canal have been rather vague. Irwin (1931) described the myenteric plexus as terminating abruptly at the level of the internal sphincter and Shimoda (1954) described it as being present in the caudal part of the rectum of the dog and monkey respectively. Shimoda also described Dogiel's types I and II cells in the myenteric plexus of the dog's rectum, but found only a small number of the second type. The nerve cells in Meissner's plexus were too primitive to enable any classification into types. However, although in the present study it was found possible to stain only type I cells in the anal canal, in the submucous plexus they were in no way immature or primitive. Type II cells stain less readily with silver and it is therefore possible that these cells do exist in the plexus in this region even though they were not detectable in my preparations.

Izumi (1955) noted abundant sensory fibres in both the zona intermedia and the zona columnaris of the anal canal and he also found 'genital nerve bodies', branched endings, and Pacinian corpuscles in human material.

Embryological studies of the anal canal have led to differing opinions concerning

the precise location of the junction between proctodaeum and cloaca. Modern text-books (Gray, 1958; Keith, 1948) locate it at the level of the anal valves, whilst others (Tenche, 1936) content themselves with the statement that the upper part of the anal canal is formed from the cloaca. Johnson (1914) considered that the 'anocutaneous line' was the junction. My studies of the sensory innervation of the anal canal support the views of Gray (1958) and Keith (1948) for there is typical somatic innervation of the epithelium as far proximal as the anal valves where, in most animals, the stratified squamous epithelium becomes simple columnar glandular epithelium. There may be some overlap proximal to the anal valves where patches of stratified epithelium are supplied by somatic nerves. This part of the problem requires further study for its elucidation.

SUMMARY

The anal canal has three zones of characteristic epithelium separated by the pectinate line and the white line (of Hilton).

The sensory nerve endings in the anal canal can be classified into three main types: free nerve endings, nerves ending in relation to hairs, and encapsulated endings.

Free nerve endings are found in the distal two zones in relation to the dermis and epidermis and also in the patches of stratified epithelium which are occasionally found more proximally.

Encapsulated endings of differing sizes and shapes exist in the submucous papillae of the pecten. They are often supplied by accessory fibres and may be interconnected by unmyelinated nerves.

No subserous plexus is present in the lower part of the rectum and the enteric plexuses here and in the upper part of the anal canal contain both Dogiel's types I and II cells. The submucous plexus in the anal canal apparently contains only type I cells.

There is no change in either plexus at the anorectal junction. The myenteric plexus can be traced without change into the pecten where it terminates at the level of Hilton's white line, sending fibres to the circular and longitudinal muscles and also between the sphincters. No neurons in Meissner's plexus are visible distal to the pectinate line and this plexus is continuous with the submucous plexus in the pecten.

The mode of innervation of the anal canal supports the view that the junction of the ectodermal derivatives is at the pectinate line.

I should like to express my thanks to Prof. G. A. G. Mitchell for suggesting the topic for research, to Dr J. R. Rintoul, Mr K. Pearson and Mr P. Howarth for technical aid, and to Mr G. Wilson and the staff of the Medical Library for their help with the literature.

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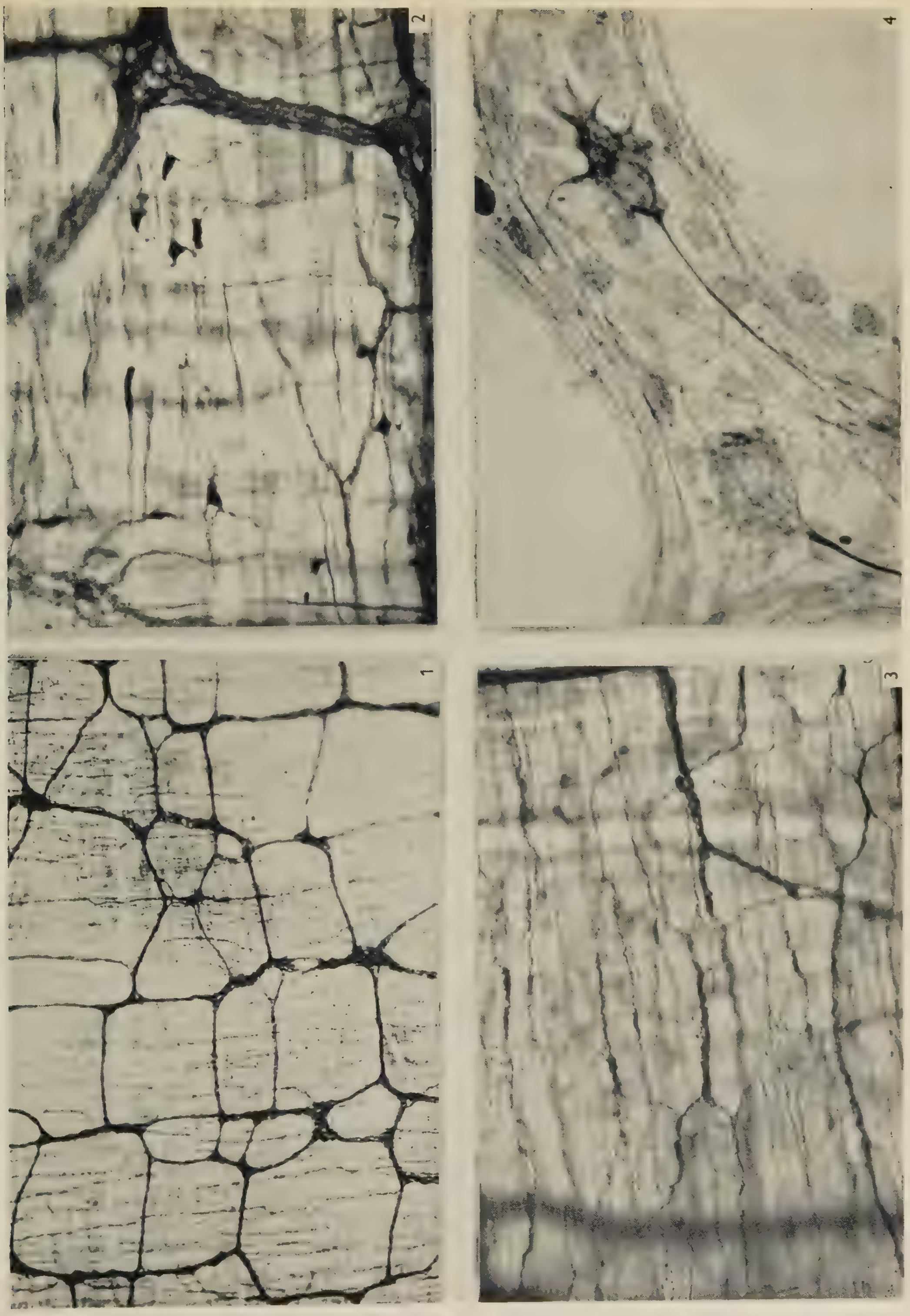

HOLMES—Intrinsic innervation of the rectum and anal canal

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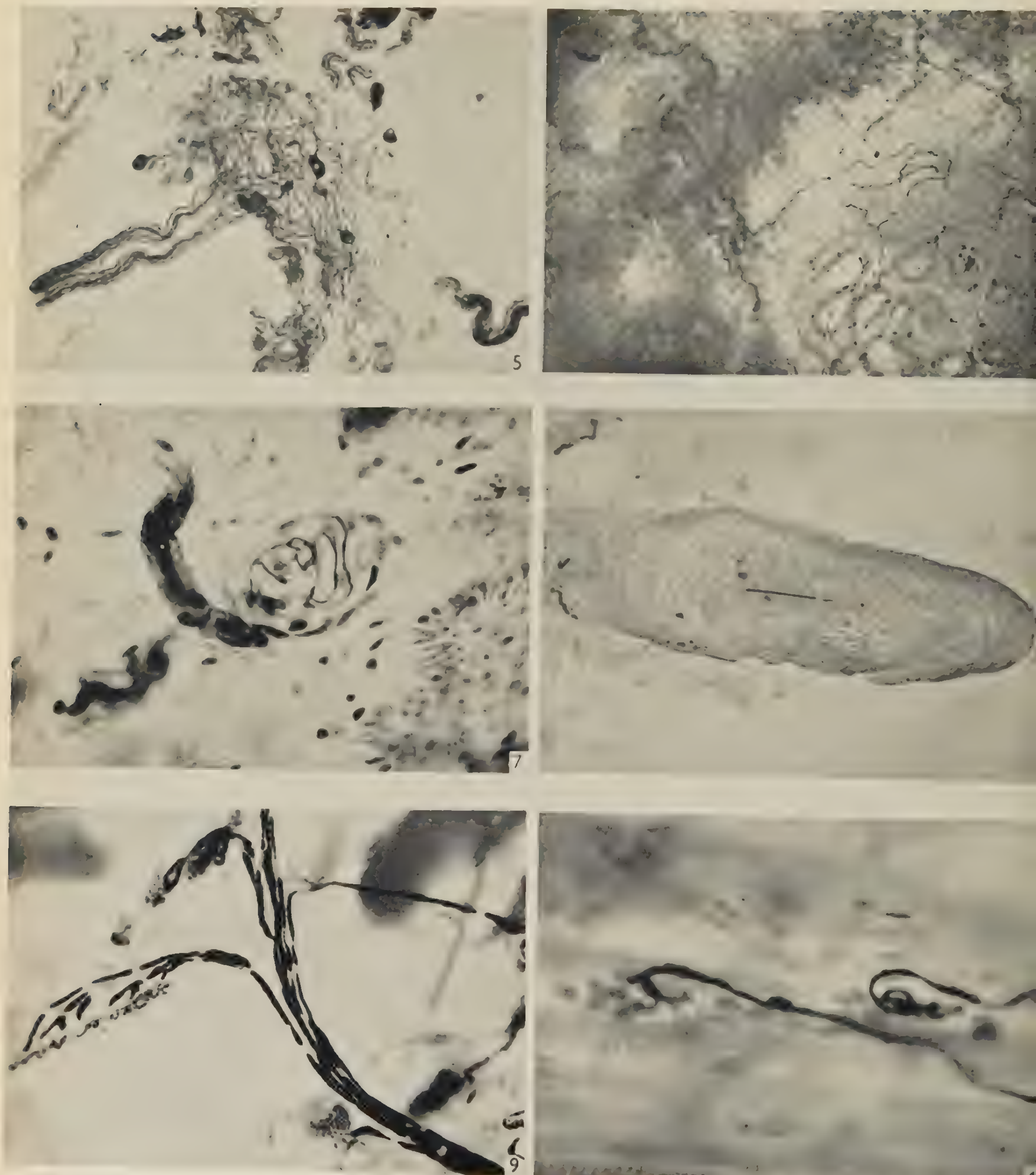

HOLMES—Intrinsic innervation of the rectum and anal canal

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Myenteric plexus in the rectum of the rat. (Supravital methylene blue. ×60.)

Fig. 2. Tertiary plexus and autonomic interstitial cells in the rectum of the guinea-pig. (Supravital methylene blue. ×550.)

Fig. 3. Subserous plexus and plexus muscularis profundus in the upper part of the rectum of the rat. (Supravital methylene blue. ×400.)

Fig. 4. Ganglion in the myenteric plexus of the rectum of the guinea-pig. (Modified Bielschowsky technique. ×450.)

Plate 2

Fig. 5. Ganglion in the myenteric plexus of the anal canal of the cat. (Modified Bielschowsky technique. ×150.)

Fig. 6. Submucous and periglandular plexus in the anal canal of the cat. (Modified Bielschowsky technique. ×150.)

Fig. 7. Encapsulated ending found in the pecten of the human. (Modified Bielschowsky technique. ×300.)

Fig. 8. Pacinian corpuscle in the pecten of the monkey. (Modified Bielschowsky technique. ×600.)

Fig. 9. Motor end plates in the external sphincter of the rabbit. (Gold chloride. ×300.)

Fig. 10. Motor end plates in the external sphincter of the cat. (Modified Bielschowsky technique. ×1100.)

ON THE DEVELOPMENT OF THE CLOACA AND THE PERINEUM AND THE FORMATION OF THE URETHRAL PLATE IN FEMALE RAT EMBRYOS

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INTRODUCTION

The process by which the cloaca is divided into two parts, a ventral part (the ventral cloacal remnant—*v.c.r.*) and a dorsal part (the dorsal cloacal remnant—*d.c.r.*), has been the subject of widely varying descriptions. Tourneux (sheep, 1888, 1894) regards the division of the cloaca as the result of a caudally growing fold between *v.c.r.* and *d.c.r.*, the mechanism behind the caudal growth of the fold being an increased growth in the entoderm connecting the *v.c.r.* and *d.c.r.* This theory has been supported by Disse (mole, 1905) and Buchanan & Fraser (marsupials, 1918). On the other hand, Lewis (man, 1911), Krasa (mole, 1918) and Henneberg (rat, 1917) are of the opinion that the connective tissue between the *v.c.r.* and the *d.c.r.* is the active factor in that this mass of connective tissue, the uro-rectal septum, grows caudally. Reichel (man, pig, 1893*a*, *b*) and Born (man, 1893) describe folds on the lateral walls of the cloaca which fuse in the median plane. According to Retterer (several species, 1890, 1893, 1905) these folds are formed by a proliferation of the epithelium in the lateral walls of the cloaca. When the folds have fused in the median plane, the intervening epithelium is transformed to connective tissue. Dimpfl (guinea-pig, 1906) assigns importance to several different factors in the division of the cloaca. Among others, he considers that the caudal part of the cloacal saccule undergoes a reduction, the entoderm connecting the *v.c.r.* and *d.c.r.* in this way coming nearer the ectoderm. Another factor of importance is a narrowing of the slender connexion between the lumen in the *v.c.r.* and *d.c.r.* ('Analrohr', Schwarztrauber, 1904). Fleischmann (1904, 1906, 1907), after discussing the mechanism behind the division of the cloaca, finally concludes that this is the result of complicated formations in the cloaca itself. Andersson (rat, 1909) regards the division of the cloaca as the result of a drawing out of the cloaca in the ano-urogenital prominence when the latter is formed.

Regarding the formation of the perineum, Tourneux (sheep, 1890) maintains that the 'repli périnéal' (the uro-rectal septum) breaks through the ectoderm and forms the perineal raphe. Politzer (man, 1931, 1932) also finds that the uro-rectal septum penetrates the cloacal membrane, thus explaining how the primary perineum comes to contain entoderm. The definite perineum is formed through this entoderm being replaced by ectoderm. Henneberg (rat, 1917) finds nothing to support a distinction between a primary and secondary perineum. This author discards the term perineal raphe as far as rats are concerned since he finds no evidence of fusion in the perineum.

Diverging views have been expressed concerning the development of the urethral plate. The present author calls the solid epithelial plate situated medially in the phallus the urethral plate. The epithelial plate connecting the lumen in the *v.c.r.*

with the surface epithelium in the phallus, after the division of the cloaca, is called the urogenital plate (Felix, 1911). Reichel (man, 1893) finds that the cloacal plate lengthens to form the urethral plate. Tourneux (sheep, 1888), Disse (mole, 1918), and Henneberg (rat, 1917) regard the urethral plate as arising through the fusion of the walls in the urogenital sinus. Schwarztrauber (pig, sheep, 1904), Siddiqi (spermophile, 1937), Glenister (man, pig, 1954, 1956), and Kanagasuntheram & Anandaraja (dog, 1960) are all of the opinion that the urethral plate is an outgrowth from the wall of the urogenital sinus. Born (man, 1893) holds quite a different view. He finds that the urethral plate has been formed through the walls of two folds merging together, and is therefore of ectodermal origin. According to Barnstein & Mossman (red squirrel, 1938) it is not the formation of folds which forms the urethral plate, but an ectodermal ingrowth along the urethral facies of the phallus.

MATERIAL AND METHODS

The material for this investigation comprises 102 embryos from albino rats, the embryos ranging in age from 9 days 12 hours post coitum (p.c.) to 20 days 6 hours p.c. After there has appeared a difference of gonadal structure of the two sexes, only female embryos have been studied. The time interval between the embryos in the material varies between 3 and 12 hr., the exactness of embryonic age determination in all cases being ± 30 min. For greater clarity in the description, some of the stages studied have been omitted in the following account. After 11 days post coitum, the embryos have been delivered by the method of successive Caesarian sections (Hjortsjö, 1945, 1946).

The routine fixative has been Bouin's fluid. Decalcification has been carried out on embryos of more than 14 days post coitum, embedding done in paraffin and 10μ sections cut transversely, and in certain cases sagittally. For staining, use has been made of a combined haematoxylin (Delafield)–eosin–orange-G stain (Bengmark & Forsberg, 1959).

On two embryos aged 17 days 6 hr. post coitum, polysaccharide reaction has been carried out by the method of Bauer (Romeis, 1946). In these cases, fixation has been done in 0·5 % chromic acid for a period of 4 days, the embryos being subsequently decalcified and embedded in paraffin in the usual manner.

An investigation has also been undertaken of the occurrence of alkaline phosphatase in the urethral plate. For this, four embryos aged 16 days have been fixed in acetone at a temperature of 4° C. From the fixation fluid, the embryos have been transferred first to alcohol-ether, then to 2 % celloidin in alcohol-ether, and subsequently to chloroform, being finally embedded in 52° paraffin, using the vacuum method, and stained according to Gomori (1946). Sodium-glycerophosphate has been employed as an incubation medium, the incubation time being 2 hr. Controls have been incubated without substrate.

For the study of the mitotic frequency, colchicine has been injected subcutaneously in a dose of 0·15 mg. into the mother. The females have been treated at 13 days 12 hr. and the embryos fixed at 13 days 16 hr.

The author has carried out Best's carmine staining after fixing in absolute alcohol on two embryos aged 14 days 16 hours.

DESCRIPTION OF STAGES

9 *days* 22 *hr.* In the caudal end of the embryo a clearly developed primitive streak can be found. Within only one section of this a distinct, relatively broad connexion between the ectoderm and the entoderm can be observed. The deeper, more mesenchyme-like cells in the primitive streak have disappeared, whilst the relatively high epithelial cells pertaining to the ectoderm are in direct contact with the somewhat lower entodermal cells. In the following account the term ecto-entodermal junction is preferred to cloacal membrane up to the time when the Wolffian ducts open into the hindgut, since before this no cloaca can be said to exist.

10 *days.* The ecto-entodermal junction has greatly increased in length, the boundary between the ectoderm and the entoderm being easily distinguishable, mainly on account of the darker colour of the ectoderm. The entodermal cell nuclei are now higher and the ectodermal cell nuclei considerably lower than earlier. Both in front of and behind the ecto-entodermal junction, as also in a narrow strip on each side of it, a transition of ectodermal cells to mesenchyme can be observed. Degeneration granules can be seen on the boundary between the distinctly ectodermal and distinctly mesenchymal cells (Pl. 1 A). Solitary granules are also present in both the ectoderm and the entoderm of the ecto-entodermal junction.

11 *days* 6 *hr.* The primitive streak remains only in the form of a narrow strip on each side of the ecto-entodermal junction. Numerous degeneration granules occur in this region in the cells lying immediately below the surface epithelium (Pl. 1 B). A large number of granules are also seen in the mesenchyme cranial to the junction between the ectoderm and the entoderm. The boundary between ectoderm and entoderm is distinct.

11 *days* 15 *hr.* The primitive streak has entirely disappeared. Degeneration granules are found in large numbers in the mesenchyme cranial to the ecto-entodermal junction, and also in some places within the latter, particularly in its cranial part.

12 *days.* At this stage, the ecto-entodermal junction extends up to the level of the umbilical cord. The boundaries between the ectoderm and the entoderm are easily distinguishable along its whole course. The anlage of the tailgut has been formed.

12 *days* 6 *hr.* The Wolffian ducts contact the lateral walls of the hindgut immediately cranial to the most cranial point of the ecto-entodermal junction. From here, they run along the lateral walls of the hindgut caudally, finally disappearing completely somewhat below the cranial part of the ecto-entodermal junction. Within the region where the ducts lie close to the hindgut, the latter is somewhat widened transversely.

12 *days* 12 *hr.* The tailgut has increased in length compared with earlier stages. Solitary degeneration granules can be observed in its ventral wall. The boundary between the ectoderm and the entoderm is distinct along the whole ecto-entodermal junction, the ectodermal cells being dark with low nuclei, whilst the entodermal cells are somewhat lighter having higher nuclei.

At this stage, the point where the Wolffian ducts gain contact with the hindgut lies relatively far caudal to the most cranial part of the ecto-entodermal junction.

One important change that has occurred at this stage is the development of an allantoic rudiment. Immediately cranial to the points of attachment of the Wolffian ducts on the lateral walls of the hindgut, a protrusion of the lumen has developed laterally on each side of the hindgut. These protrusions become more and more pronounced in a cranial direction. It can be observed, at the height of the most cranial point of the ecto-entodermal junction, how the lateral walls of the hindgut dorsal to the protrusions curve in and finally meet in the median plane. This produces a dorsal and a ventral lumen. In more cranial sections the dorsal and the ventral lumen are completely separated by mesenchyme. The arrangement of the mesenchyme within this area, and in the region where the walls of the hindgut curve in, gives the impression that it participates actively in the above-mentioned division of the hindgut. Degeneration granules can be observed in the incurving epithelium and in the common epithelial wall between the ventral and the dorsal lumen.

12 *days* 21 *hr.* The ecto-entodermal junction no longer extends up to the lower edge of the umbilical cord. The entrance of the allantoic rudiment into the hindgut is level with the most cranial point of the ecto-entodermal junction. As mentioned above, the lateral walls of the hindgut immediately caudal to this orifice curve in. Degeneration granules are found in the epithelium within this region and in the common wall, existing in a few sections, between the lumen of the allantoic rudiment and that of the hindgut (Pl. 2). No degeneration granules are found in the hindgut dorsal to the incurvation.

In the tailgut, a marked increase has taken place in the number of degeneration granules, these occurring most abundantly in the ventral wall. Numerous degeneration granules occur at the points of attachment of the Wolffian ducts on the walls of the hindgut, caudal to the cranial end of the ecto-entodermal junction.

13 *days.* A patent connexion has arisen between the Wolffian ducts and the hindgut in the form of slitlike openings, around which degeneration granules can be observed. These orifices are situated level with the cranial end of the ecto-entodermal junction. Since the Wolffian duct and intestine thus open from this stage onwards into a common lumen, this will now be called the cloaca. Similarly, the term 'ecto-entodermal junction' is replaced by the term 'cloacal membrane'. As it is extremely difficult in early stages to distinguish the boundaries between the allantois, the bladder anlage, the primitive urethra, and the urogenital sinus, the present author makes use of the term 'the ventral cloacal remnant', *v.c.r.*, as a comprehensive term for these structures. The dorsal part of the divided cloaca is called 'the dorsal cloacal remnant', *d.c.r.* As in earlier stages, the boundary between the ectoderm and the entoderm in the cloacal membrane is distinct.

In a few sections caudal to the opening of the *v.c.r.* into the cloaca, a depression containing degeneration granules is observed in the epithelium on the lateral walls of the cloaca. Caudally, these granules appear on the dorsal wall of the cloaca and on the immediately adjacent parts of the lateral walls. At the caudal end of the cloacal membrane, the granules are localized only in the dorsal wall.

The tailgut has diminished considerably compared with earlier stages, large numbers of degeneration granules being present in the remaining part.

13 *days* 9 *hr.* The distance has increased between the cranial end of the cloacal

membrane and the umbilical cord. Moreover, the cloacal membrane has now become transformed into a sagittally disposed plate. Solitary degeneration granules can be seen in the entodermal part of this plate. The above-mentioned difference in colouring between the ectoderm and the entoderm is still striking. The relatively high entodermal cell nuclei contrast also in form with the low ectodermal cell nuclei. A large number of degeneration granules occur in the caudal part of the membrane.

In this stage, the orifices of the Wolffian ducts lie on the *v.c.r.* cranial to the most cranial point of the cloacal plate. In the epithelial connexion between the *v.c.r.* and the *d.c.r.* there is now a substantially smaller number of degeneration granules than earlier. On the other hand, the number of granules has greatly increased in the dorsal wall of the cloaca and also to a lesser degree in the adjacent parts of the lateral walls. Solitary granules are also observed in the most caudal part of the *d.c.r.* The tailgut has now disappeared completely.

13 *days* 15 *hr*. The boundary between the ectoderm and the entoderm in the cloacal plate is distinct. In the caudal part of the latter, degeneration granules are found in the entoderm. The division of the cloaca has progressed considerably further than in the preceding stage. Degeneration granules are found in large numbers in the dorsal and immediately adjacent parts of the lateral walls of the cloaca.

14 *days*. Degeneration granules can no longer be observed in the cloacal epithelium connecting the *v.c.r.* with the *d.c.r.* On the other hand, these granules do occur in large numbers in the dorsal parts of the lateral walls of the cloaca. The cloacal plate is now wider transversely in its cranial part than in the preceding stage. Furthermore, it is somewhat more difficult to distinguish the boundary between the ectoderm and the entoderm in the most cranial part than in other regions of the plate. The genital tubercle is now clearly developed. From now on the term 'apical' will be used to indicate a position nearer the apex of the genital tubercle and 'basal' to indicate a position nearer the root. The side of the genital tubercle along which the cloacal plate is attached is called the urethral facies; the opposite, the dorsum.

14 *days* 6 *hr*. The entoderm in the most apical, wider part of the cloacal plate contains degeneration granules. On the urethral facies immediately apical to the cloacal plate, a slight depression can be observed extending a few sections in an apical direction.

14 *days* 15 *hr*. Large numbers of degeneration granules appear, as described above, in the dorsal wall of the cloaca. Solitary granules can be seen in the most caudal part of the *d.c.r.* and also in the most basal part of the cloacal plate.

At the apical end of the cloacal plate, where the latter is transversely broader than in other regions, the entoderm protrudes in the form of a swelling covered by a thin layer of ectoderm, the boundary between the two types of epithelium being relatively distinct. This entodermal protrusion contains a large number of degeneration granules. An interesting phenomenon appearing at this point is the formation in a few sections apical to the cloacal lumen of a solid reduplication consisting of two cell layers and appearing to form a direct continuation of the walls of the cloacal lumen. In the author's view, this reduplication appears to have developed in the following manner: in the most apical part of the genital tubercle, no basal membrane

is visible under the surface ectoderm after routine staining. Somewhat more basally, however, a basal membrane does occur within a smaller area on the urethral facies. After a few more sections in a basal direction, the ectodermal cells in the region of the basal membrane assume a somewhat higher form than in other parts of the surface ectoderm. Still further basally, these higher cells begin to curve inwards into the mesenchyme, but their staining capacity is the same as in other parts of the surface ectoderm. Finally, the incurving ectodermal cells form a reduplication which is stained somewhat lighter than the surface epithelium. The epithelium in the reduplication passes without any boundary both into the surface ectoderm and into the epithelium around the lumen of the cloaca. Along its superficial margin, the reduplication is not covered by the squamous epithelial cells found in other places in the surface ectoderm. These first appear level with the above-mentioned protuberance. This indistinct transition between the ectoderm and the entoderm is in strong contrast to the distinct boundary still existing between the two types of epithelium in the cloacal plate. The reduplication described here constitutes the first rudiment of the urethral plate.

15 *days*. Degeneration granules still occur in the dorsal cloacal wall, which is now quite short. The urethral plate has increased in length; it is broadest in its apical part and gradually narrows in the basal direction. In the more apical part, degeneration granules occur immediately adjacent to the urethral facies. The relation between the ectoderm and the urethral plate is the same as mentioned above. Along the cloacal plate it is relatively easy to distinguish the boundary between the ectoderm and the entoderm. The squamous epithelium of the surface ectoderm is lacking in the most apical part of the reduplication, but it appears in more basal sections. Numerous degeneration granules can be observed in the mesenchyme around the apical part of the urethral plate (Pl. 4).

15 *days* 6 *hr*. The division of the cloaca has now progressed so far that one can no longer speak of any real cloacal lumen. The lumina in the *v.c.r.* and in the *d.c.r.* are connected to one another by means of a narrow canal, the so-called 'Analrohr' (Schwarztrauber, 1904), which runs in the dorsal part of a plate consisting of two cell layers, the so-called 'perineophallic lamina' (Henneberg, 1917). This is an entodermal lamina connecting the *v.c.r.* with the *d.c.r.*, a remnant of the earlier cloacal plate within this region, which will later develop into perineum. Around the edges of the Analrohr are degeneration granules. As the cloaca no longer exists, the part of the cloacal plate corresponding to *d.c.r.* is now called the anal membrane; the part corresponding to the lumen of the *v.c.r.* is called the urogenital plate. The perineophallic lamina is situated between these two structures.

The impression is that the above-mentioned protuberance, which contains large numbers of degeneration granules, moves in an apical direction at the same speed as the broad, most apical part of the urethral plate is transformed to a narrow one.

15 *days* 12 *hr*. The boundary between the ectoderm and the entoderm is distinct along the whole perineophallic lamina. In that part of the lamina lying nearest the *d.c.r.*, the entoderm has lost its ectodermal covering. Solitary degeneration granules are found in the ectoderm bordering the lamina laterally. A large number of granules are seen in the ectoderm covering the lamina apical to the point where the perforation of the ectoderm has taken place.

16 *days.* The development of the urethral plate continues in the manner described earlier. The perineophallic lamina has completely disappeared in the immediate vicinity of the *d.c.r.* Only a strip of entoderm remains, situated in the ectoderm without being covered by it. The lamina reappears in an apical direction, becoming progressively higher. Degeneration granules can be seen both in the part of the lamina lying immediately adjacent to the ectoderm and in the apical part, where they occur in large numbers. No granules are found in the dorsal part of the lamina, nor in the entodermal strip in the ectoderm.

16 *days* 15 *hr.* The disappearance of the ectoderm from the perineophallic lamina continues in an apical direction. The occurrence of degeneration granules is the same as in the previous stage.

The urethral plate continues to grow in an apical direction. Here the same appearances described earlier in the development of this structure persist. Apically the cell nuclei of the basal layer of the ectoderm change their form from almost cubical to cylindrical. The ectoderm forms a double-layered cell plate extending into the mesenchyme and passing without a boundary into the other epithelium in the urethral plate. Degeneration granules are present both in the wide apical end of the plate and in the mesenchyme around its most apical part. The boundary between the ectoderm and the entoderm is distinct in the urogenital plate, but there is a gradual transition in the urethral plate. No squamous epithelial cells occur in the surface epithelium along the most apical part of the urethral plate.

17 *days* 6 *hr.* In the perineum, a few sections apical to the orifice of the *d.c.r.*, it is now impossible to distinguish any particular epithelium, since all the cells have an ectodermal appearance. In an apical direction, there is a gradual transition to a strip of typical entodermal cells in the middle of the perineum (Pl. 5). The cell nuclei in both the entoderm and the ectoderm are of the same size, but the entodermal cytoplasm is considerably paler than the ectodermal. Still further apically, degeneration granules can be seen in the ectoderm bordering the entodermal strip laterally. Apical to this area is found the beginning of the rest of the perineophallic lamina, which is covered in its most apical part by a thin layer of ectoderm, containing a large number of degeneration granules. Granules occur also in the part of the lamina lying in the immediate proximity of the ectoderm, whilst the dorsal part of the lamina is completely free of them.

17 *days* 18 *hr.* Of the perineophallic lamina there now remains only a small remnant in the most apical part of the perineum. The incorporation of entodermal cells in the perineum has progressed in an apical direction as described above.

It is interesting to study the boundary between the ectoderm and the entoderm. Along the urogenital plate, the cells in the basal layer of the ectoderm are stained darker than those in the entoderm, whilst the size of the nuclei in the two types of the epithelium is approximately the same. Along the whole of the urethral plate, on the other hand, no difference can be observed in staining capacity, the basal layers in the two epithelia passing imperceptibly into one another. In the most apical part of this structure, both the occurrence of degeneration granules and the epithelial conditions are as described earlier.

18 *days* 6 *hr.* The perineophallic lamina has now disappeared. However, in the most apical part of the perineum, traces can still be found of entodermal cells lying

in the ectoderm. Degeneration granules are also found in the laterally adjacent ectoderm. Along the whole perineum, there are in the median line no squamous epithelial cells in the surface epithelium, thus corresponding to the region of the incorporated entodermal cells, which, however, with the exception of the most apically situated, have now the appearance of ectoderm.

18 *days* 18 *hr*. In the apical part of the perineum, there is still no squamous epithelial covering in the median line. Cells with entodermal appearance or degeneration granules no longer occur within this area.

In contrast to earlier stages, the most apical part of the urethral plate is also covered by squamous epithelium. At its most apical point the plate is broad, but its sagittal height increases gradually in a basal direction. The earlier-mentioned protuberance in the apical area of the urethral plate has completely disappeared. Only solitary degeneration granules now occur in the broad part of the plate: a great reduction has similarly taken place in the number of granules in the mesenchyme around the cranial part of the plate.

19 *days* 15 *hr*. It is considerably more difficult to distinguish the boundary between the ectoderm and the epithelium lying inside it in the urethral than in the urogenital plate. However, the cytoplasm of the ectodermal cells is more blurred than that of the cells in the urethral plate, where it is clear. Moreover, the cells in the basal layer of the surface ectoderm are somewhat more darkly stained than the basal cells in the plate. These characteristics are more pronounced in the basal part of the urethral plate than in the apical part. In the first-mentioned area, the basal cell nuclei in the surface epithelium are somewhat higher than the same cell nuclei in the plate. Solitary degeneration granules are present in the most apical part, as also in the mesenchyme around it.

20 *days* 6 *hr*. The entire perineum is now covered by squamous epithelium. Degeneration granules can no longer be seen either in the most apical part of the urethral plate or in the mesenchyme in this region.

DISCUSSION

The first appearance of a junction between the ectoderm and the entoderm in the posterior end of the body, the rudiment of the cloacal membrane, is located in the region of the posterior part of the primitive streak. However, it is of interest that the author has been able to observe degeneration granules in the primitive streak during the later stages of its existence, from approximately 10 days to 11 days 6 hr., after which all traces of it disappear. A possible explanation for the appearance of the granules within this area is that the cells, which are in a stage of differentiation between ectoderm and mesenchyme, when the primitive streak ceases to function, might not be able to complete their differentiation but die. Such an interpretation makes unlikely Zuckerman's (1940, 1950) assumption of the retention of a latent capacity for proliferation by the ectoderm within this area of the primitive streak. Moreover, it has not been possible to observe any ectodermal ingrowth in the *v.c.r.* as described by Burns (opossum, 1942) and later by Walz (man, 1959). The boundary between the ectoderm and the entoderm is distinct in the urogenital plate during the whole period studied, even after the *v.c.r.* establishes a communication outwards.

The ecto-entodermal junction increases in extent and reaches finally up to the lower edge of the umbilical cord. This increased growth is accompanied by an abundant occurrence of degeneration granules in the mesenchyme between the ectoderm and the entoderm, cranial to the point of junction before the latter has reached the level of the umbilical cord. In the author's opinion, this indicates that the interjacent mesenchyme degenerates while the entoderm continues to gain contact with the ectoderm in a cranial direction. The solitary granules, which can be seen in the ecto-entodermal junction, are possibly resorbed granules from the degenerated mesenchyme. After the 12 day 12 hr. stage, the ecto-entodermal junction no longer reaches the level of the umbilical cord.

Between the stages 13 days 9 hr. and 13 days 15 hr., the cloacal membrane (earlier the ecto-entodermal junction) is transformed into a sagittal plate, the cloacal plate. This transformation is accompanied by an occurrence of degeneration granules in the entodermal part of the cloacal membrane.

When the embryos are 12 days 12 hr. old, the first allantoic rudiment appears. At the point where this opens into the hindgut, numerous degeneration granules can be seen in the common wall, existing in a few sections, between the already mentioned rudiment and the hindgut. Degeneration granules also occur in the incurving epithelium on the lateral walls of the hindgut caudal to the orifice. This occurrence of degeneration granules indicates that at least the first division of the hindgut and later the cloaca, is produced by the walls of the former curving in, until they meet in the median plane and that this is followed subsequently by degeneration of the interjacent epithelium. The arrangement of the mesenchymal cells between the allantoic rudiment, and later the *v.c.r.*, on the one hand, and the hindgut, and later the *d.c.r.*, on the other hand is such that they can be suspected of taking an active part in the development of the grooves on the lateral walls of the hindgut. This is also supported by the abundant occurrence of mitoses in the mesenchyme within the region of the grooves.

At 13 days the degeneration granules have begun to appear in the dorsal wall of the cloaca and in the immediately adjacent parts of the lateral walls. Whilst these granules rapidly increase in number here, they disappear more and more from the common wall between the *v.c.r.* and the *d.c.r.*, and also from the regions of the grooves, which gradually regress. Within the mentioned areas, only isolated granules are found after 13 days 15 hr. The degeneration granules increase in the dorsal and lateral walls of the cloaca, with the result that this area finally becomes the one where the largest number of granules is found during the entire development of the genital sphere in rat, a fact which points to a particularly extensive degeneration. During the subsequent division, solitary granules are also found in the most caudal part of the *d.c.r.* Here it is probably a case of granules being resorbed from the strongly degenerative epithelium lying caudal to this area. The degenerative conditions observed argue strongly in favour of the division of the cloaca occurring mainly in the manner described by Dimpfl (1906). However, this author does not speak of any degenerative processes in this region. The result of the described degeneration appears to be an extensive shortening of the dorsal wall of the cloaca. In addition, it has been possible to show an increased growth in the most caudal part of the *d.c.r.* Mitotic counts in rat embryos treated with colchicine reveal

that the mitotic frequency within this region is higher than in the more cranial part of the *d.c.r.* ($t = 5{\cdot}10$ for 10 D.F., $P < 0{\cdot}001$). It seems to the author that the connecting epithelium between the *v.c.r.* and *d.c.r.* can, as a result of this growth, move caudally, forming the dorsal wall of the *v.c.r.* In this, only isolated mitoses are encountered. As a result of this growth and the degenerative processes, the intestinal orifice finally reaches the surface of the body at 15 days 6 hr. After this there remains, however, a narrow connexion between the lumen in the *v.c.r.* and in the *d.c.r.*, the 'Analrohr'. Around the Analrohr, degeneration granules are found in the epithelium, indicating that the epithelium around the Analrohr is degenerating and will not be included in the solid reduplication lying immediately ventral to it and connecting the *v.c.r.* with the *d.c.r.*, i.e. the perineophallic lamina (Henneberg, 1917).

It is interesting to note how the entoderm in the perineophallic lamina in rat, as also in man (Politzer, 1931, 1932), and sheep (Tourneux, 1888), breaks through the surface ectoderm which is here undergoing a marked degeneration. The perineophallic lamina, which was in earlier stages relatively high, now becomes lower, partly on account of a degeneration of its ventral part and partly, probably, also on account of an extension when the perineum increases in length. In the dorsal part of this lamina, on the other hand, degeneration granules are never found. It is just this dorsal part that comes to lie as a median strip in the ectodermal epithelium in the perineum. It is then possible to follow how this entodermal epithelium differentiates, beginning in the area near the *d.c.r.*, and becoming more and more like ectodermal epithelium. No degeneration granules are found in the entodermal strip during this differentiation. The last entodermal characteristic the cells lose is the clear cytoplasm and thus they also lose their capacity for red staining in the polysaccharide reaction of Bauer, by which they are distinguished earlier (Pl. 6). When this capacity disappears, the entodermal strip is covered by squamous epithelium and no epithelium differing from the surrounding ectoderm in the perineum can any longer be distinguished. Henneberg (rat, 1917) has noticed the lack of squamous epithelium in the median part of the perineum but does not mention an entodermal penetration of the ectoderm. No real reason exists for speaking of a primary and a definitive perineum in the rat, since the incorporated entoderm does not disappear as in man (Politzer, 1931, 1932), but merely undergoes a metaplasia.

Concerning the derivation of the urethral plate, the author finds a number of reasons in support of its ectodermal origin. From the earliest to the last embryonic stage studied in which a junction between ectoderm and entoderm is found the boundary is always distinct. The boundaries between the ectoderm and the epithelium in the urethral plate, on the other hand, are found throughout to be fluid. Only in the last stages studied are the boundaries somewhat more pronounced. The present author finds the urethral plate to be formed, therefore, in a manner closely corresponding to that described by Barnstein & Mossman (1938). Somewhat apical to the urethral plate, a distinct basal membrane is formed within a small region, whilst such a membrane is not seen in ordinary routine stainings under the ectoderm in other regions on the same level. Further basally, the ectodermal cell nuclei in the same region become somewhat higher than those around them. Finally, in a basal direction, the ectodermal cells curve into the mesenchyme in the form of a

reduplication. The degeneration granules found around the incurving epithelium can probably be accepted as a sign of the mesenchyme here having to give way to the incurving ectodermal cells. After curving into the mesenchyme in the form of the reduplication, the ectodermal cells differentiate and come more and more to resemble those found around the walls of the *v.c.r.* In its most apical part, the urethral plate is transversely broad, but it becomes increasingly narrower in a basal direction at the same time as the sagittal height increases. Degeneration granules are found in the broad part of the plate somewhat basal to its most apical part, the presence of the granules here probably being associated with the change from a transversely broad to transversely narrow plate in this region. The gradual transition between the ectoderm and the epithelium in the urethral plate is distinct both in transversely and sagitally sectioned material. In the most apical part, where the ectoderm curves into the mesenchyme, there are no squamous cells in the surface ectoderm. They do appear, however, in adjacent parts of the ectoderm. Neither in the ectoderm nor in the mesenchyme apical to the plate are any degeneration granules encountered. Kanagasuntheram & Anandaraja (dog, 1960) claim to have found a larger number of mitoses in the anterior wall of the *v.c.r.* than in other parts of this region, taking this as a support for the urethral plate becoming formed as a lamellar outgrowth from the anterior wall of the *v.c.r.* The present author has not been able to confirm this observation in the rat. The highest mitotic frequency at 16 days is found in the most apical part of the plate. Thereafter the mitotic frequency decreases very rapidly in a basal direction, being very low in the region where degeneration granules are found, that is, the broad apical part. Basally, the mitotic frequency increases in the region where the plate increases its sagittal height, in order again to decrease in the most basal part of the plate and in the apical region of the lumen in the *v.c.r.* A further argument supporting the ectodermal origin of the urethral plate is constituted by its content of alkaline phosphatase. The ectoderm and the most apical part of the plate, where the ectoderm has newly curved in, are free from this enzyme (Pl. 7). In a basal direction, however, it is possible to trace an increasing concentration of phosphatase, a fact which results in the plate finally becoming stained completely black. The urogenital plate is also stained with the same intensity. This indicates that the incurving ectodermal cells, disregarding their purely morphologic appearance, have come more and more to resemble the entodermal cells.

Henneberg (1914) has shown in the ecto-entodermal junction in early stages the presence of granules which, according to him, can be stained with Best's carmine. Disse (1905) calls the same bodies 'chromatophile granules' and is of the opinion that they occur only in the ecto-entodermal junction. Henneberg, however, disagrees with Disse on this point, since he has found them in other regions also, e.g. in the lumen of the cloaca and around the openings of the Wolffian ducts into the *v.c.r.* The author has carried out Best's carmine staining on rat embryos aged 14 days 16 hr. which shows that the degeneration granules described are not stained red (see also Bengmark & Forsberg, 1959).

SUMMARY

The author has studied 102 female rat embryos aged between 9 days 12 hr. post coitum and 20 days 6 hr. post coitum. The embryos have been sectioned both transversely and sagitally in sections of 10μ. Use has been made of a combined stain consisting of haematoxylin (Delafield)–eosin–orange-G. In addition, special stainings have been carried out, these being the polysaccharide reaction of Bauer and the alkaline phosphatase reaction. Colchicine treatment of the mother has been employed for the purpose of studying the mitotic frequency in the cloaca.

The author has shown that a degeneration occurs in the primitive streak during the last period of its existence. It is for this reason unlikely that the ectoderm maintains a latent capacity for proliferation within this region.

Only the first phase of the division of the cloaca is produced through lateral mesenchymal folds. The greatest part of the division is caused by a marked degeneration in the dorsal and adjacent parts of the lateral walls of the cloaca, together with an increased growth in the caudal part of the *d.c.r.*

Incorporated in the perineum is a median strip of entoderm which later becomes ectodermal in appearance.

A number of factors argue in favour of the urethral plate being of an ectodermal origin: the gradual differentiation of the ectoderm apical to the plate during the whole of the development of the latter and also the incurving of this epithelium into the mesenchyme of the genital tubercle. Furthermore, the mitotic frequency in the urethral plate is highest in its most apical part. After staining according to Gomori, alkaline phosphatase is not found in the most apical part of the urethral plate; it does occur, however, in increased amount in a basal direction. In the author's opinion, the urethral plate is of an ectodermal origin, although these ectodermal cells later assume more and more the same character as the entoderm around the walls of the *v.c.r.*

No ectodermal ingrowth in the *v.c.r.* has been observed.

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EXPLANATION OF PLATES

Plate 1

A. Rat 10 days. Solitary degeneration granules can be seen within the area of the primitive streak. B. Rat 11 days 6 hr. A large number of degeneration granules can be observed within the area of the remaining part of the primitive streak. *hg.*, hindgut. Magnification: approx. ×895.

Plate 2

A. Rat 12 days 21 hr. A cross-section through the caudal part of the embryo. The arrow indicates the region which is seen at a high magnification in B. Magnification approx. ×75. B. Degeneration granules in the region of the incurvation on the lateral walls of the hindgut. Magnification: approx. ×1700

Plate 3

Rat 14 days. A shows a cross-section through the caudal part of the embryo. The region of the cloaca within the square is shown in higher magnification in B and C. Numerous degeneration granules occur around the dorsal and immediately adjacent parts of the walls of the cloaca, as can be seen in both B and C. B is the more cranial section of the two. Magnification: A approx. ×14; B and C approx. ×755.

Plate 4

Rat 15 days. A shows a cross-section through the apical part of the genital tubercle. The region within the square is shown in higher magnification in B–G. B is the most apical section, G the most basal one. In B a basal membrane has formed below the surface epithelium in the region in question; in C and D the surface epithelium has begun to curve into the mesenchyme of the phallus; simultaneously, the cell nuclei in the incurving part become higher than those in its surroundings. In E mitoses are observed in the ectoderm bordering the urethral plate. F shows the violent degeneration occurring in the mesenchyme around the apical part of the urethral plate. In G, finally, degeneration granules are observed in the urethral plate itself in the region where it passes from being broad to becoming narrow more basally in a transverse direction. This figure is orientated with the deeper part of the urethral plate to the right. Magnification: A approx. ×14; B–G approx. ×930.

Plate 5

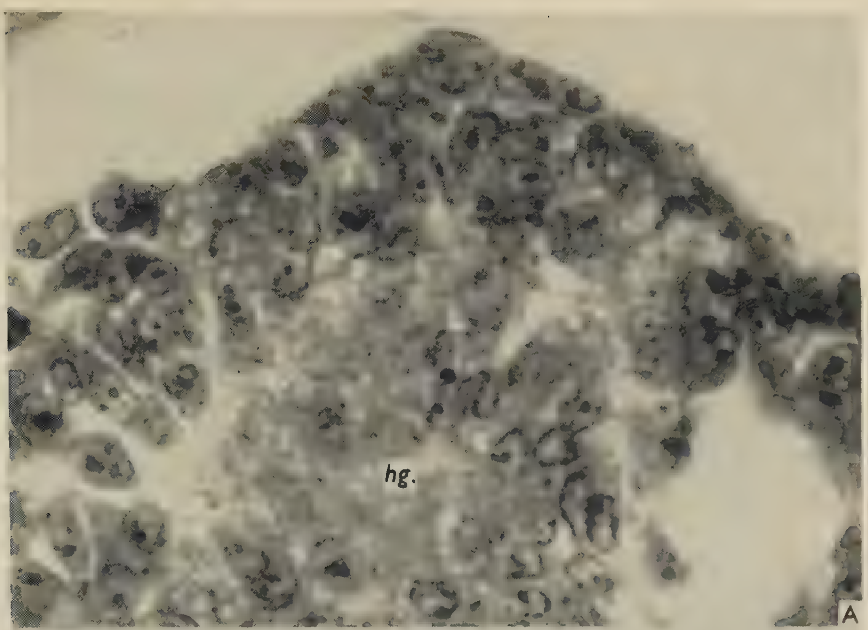
Rat 17 days 6 hr. A shows the epithelium in the perineum immediately apical to the orifice of the *d.c.r.* Here, no epithelium deviating from the surroundings can be distinguished with any certainty. In B, however, somewhat apical to A, a strip of cells is seen with lighter cytoplasm and nuclei in the median line. This strip is the last observable remnant of the perineophallic lamina. Solitary degeneration granules are seen on the boundary between the epithelium in the strip, the entoderm, and the surrounding ectoderm. Magnification: approx. ×560.

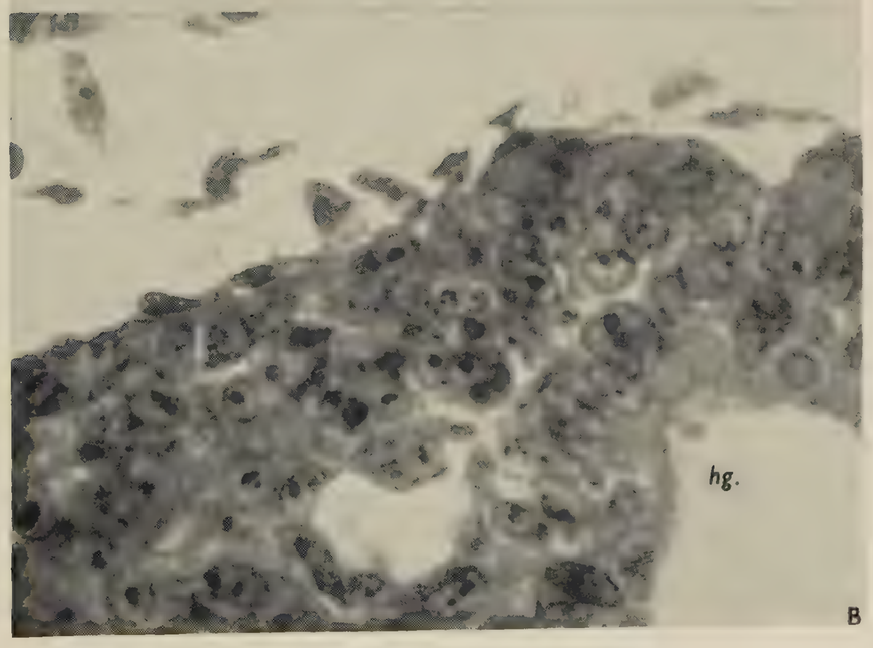
Plate 6

Rat 17 days 6 hr. Polysaccharide staining according to Bauer. Only in the median part of the perineum, within the region where an entodermal strip is incorporated in the ectoderm, is there a positive reaction to polysaccharides. Magnification: approx. ×285.

Plate 7

Rat 16 days. Staining of alkaline phosphatases according to Gomori. Although the most apical part of the urethral plate, seen in A, is free from these enzymes, they do occur more basally in the plate. Magnification: approx. ×15.





FORSBERG—Development of the cloaca and the perineum

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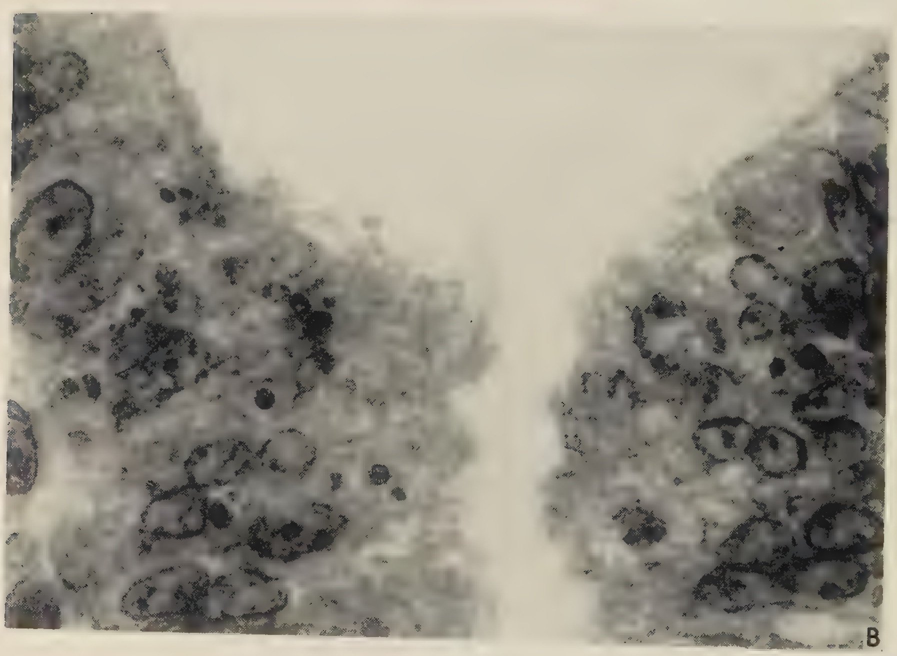

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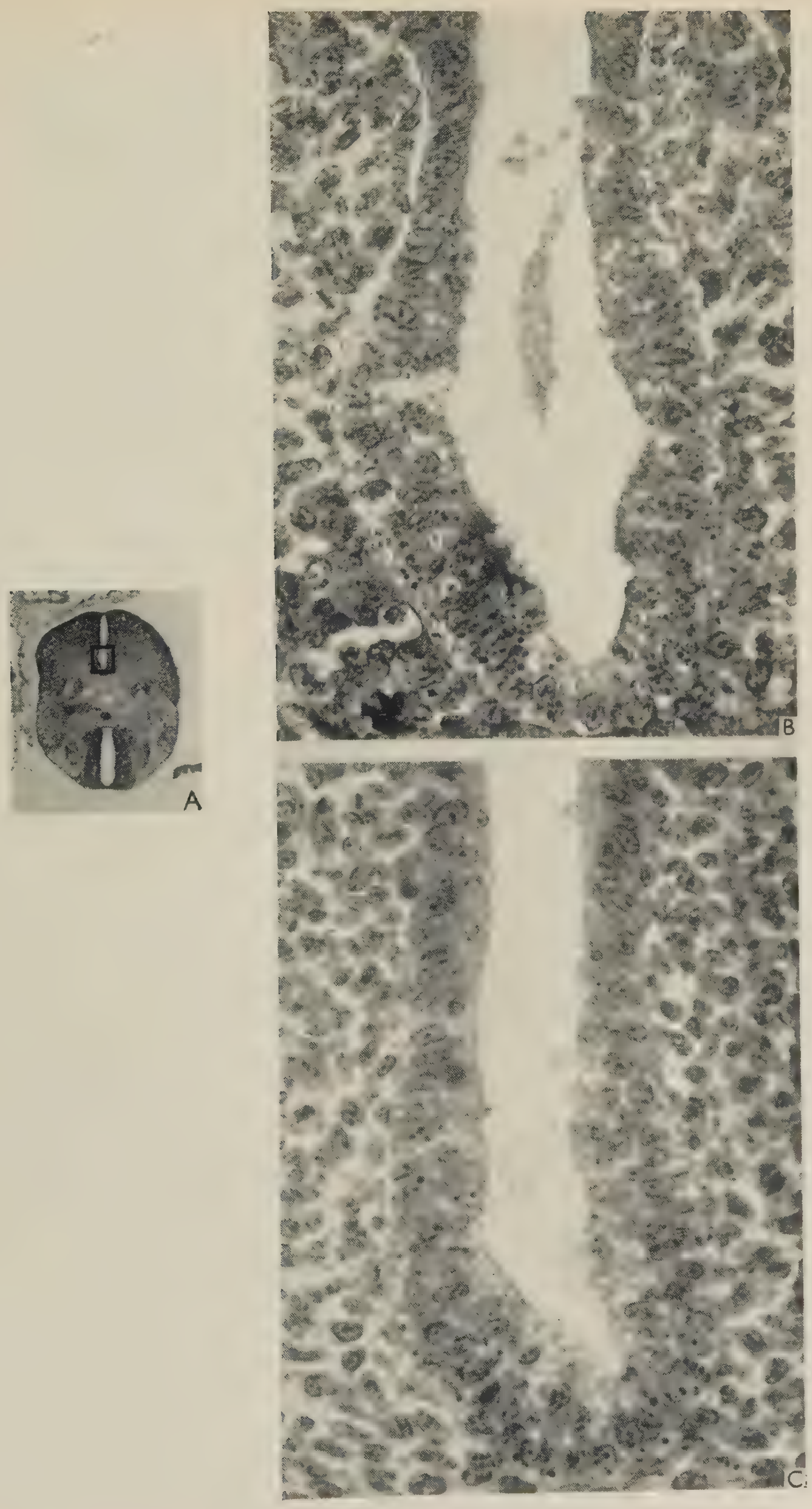

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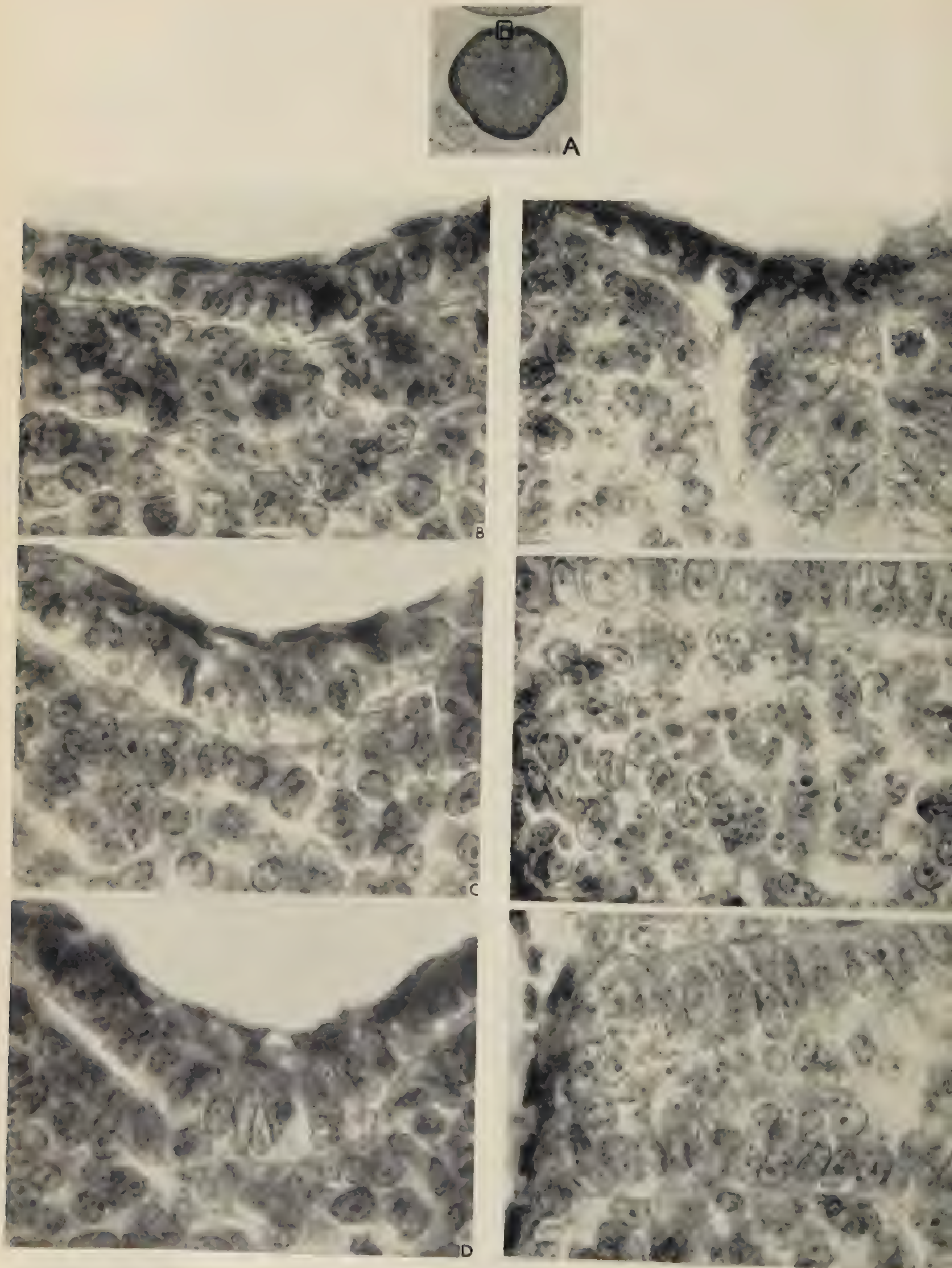

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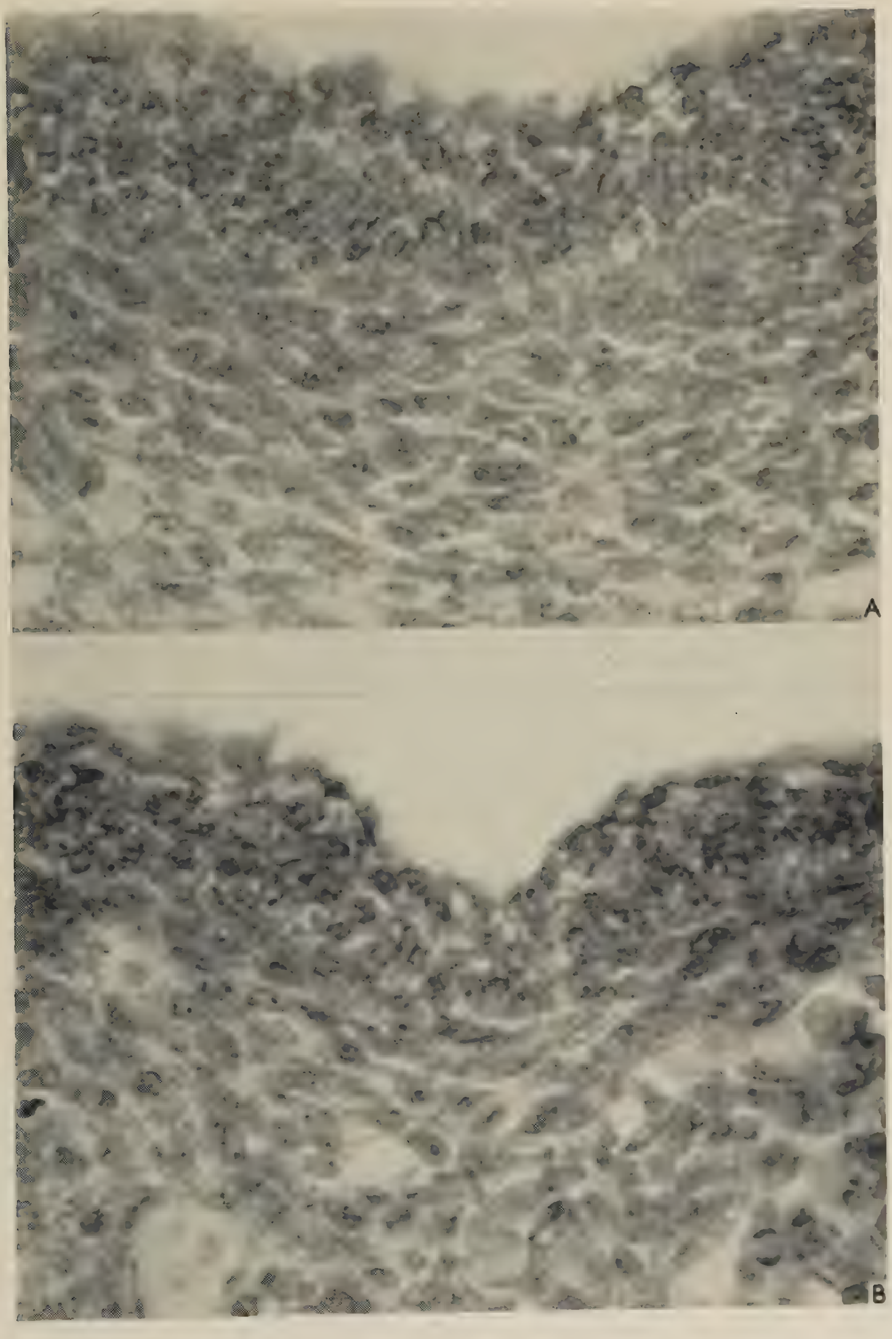

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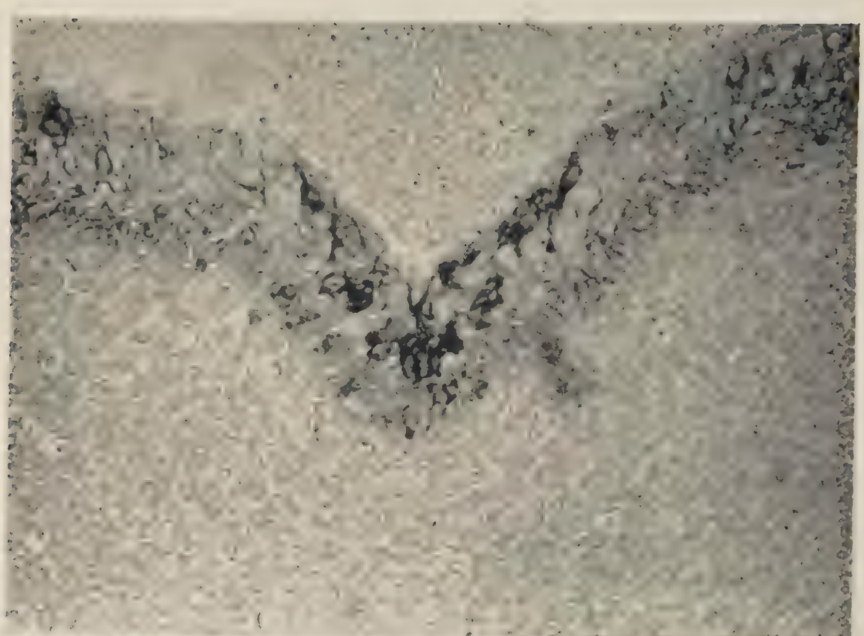

FORSBERG—Development of the cloaca and the perineum

FORSBERG—DEVELOPMENT OF THE CLOACA AND THE PERINEUM

LORD STOPFORD OF FALLOWFIELD

(*Facing p.* 437)

IN MEMORIAM

LORD STOPFORD OF FALLOWFIELD, K.B.E., M.A., M.D., Sc.D., D.Sc., LL.D., D.C.L., F.R.C.S., F.R.C.P., F.R.S.

John Sebastian Bach Stopford was born at Hindley Green, near Wigan, on 25 June 1888 and he died in his sleep on 6 March 1961, at Arnside, Westmorland.

He was educated at Liverpool College and Manchester Grammar School and in 1906 he became a medical student at Manchester University. During his undergraduate career he gained a number of distinctions and prizes and he graduated M.B., Ch.B. with Honours in 1911. After serving as a House Surgeon at Rochdale Infirmary and as an Assistant Medical Officer at Manchester Royal Infirmary he became in 1912 a Junior Demonstrator in Anatomy under Prof. (later Sir) Grafton Elliot Smith in his Alma Mater. Thereafter he became a Senior Demonstrator and in 1915 he was promoted to a Lectureship. In the same year he received the degree of M.D. with a gold medal for a thesis of outstanding merit on 'The arteries of the pons and medulla'. At this time and until the end of the First World War he also served as neurologist in turn to the Second Western General Hospital and the Grangethorpe Hospital where he started his researches on sensation; his services were recognized by the award of the M.B.E.

Following Elliot Smith's departure to University College Hospital, London, J. S. B. Stopford was appointed to succeed him (1919) as Prof. of Anatomy in Manchester at the early age of 31. His students had been so impressed by his energy, personality and teaching ability that they organized spontaneously a mass testimonial in support of his election. Five years ago a later generation of students founded an annual 'Stopford Lecture' in his honour, and it is interesting that the only other such lecture founded by the Medical Students' Representative Council is in memory of Frederick Wood Jones. 'Jock' as he was known to all, and that was a true measure of his popularity and humanity rather than of familiarity or disrespect, was invited on three occasions to accept other anatomical chairs, but he preferred to remain in Manchester.

During his period as professor he twice acted as Dean of the Medical School (1923–27 and 1931–33) and from 1928–30 he was Pro Vice-Chancellor. In 1934 when the then Vice-Chancellor, Sir Walter Moberley, left Manchester to become Chairman of the University Grants Committee, Stopford was appointed temporary Vice-Chancellor for six months 'until permanent arrangements are made'. Stopford rose to his new responsibilities with such conspicuous success, however, that neither Senate nor Council had any doubts that he was the best man and they and the University Court confirmed him in the post which he adorned for the next 22 years. By common consent he was the finest Vice-Chancellor Manchester University has ever had and Sir Hector Hetherington, Principal of Glasgow University, described him in 1956 as 'the best of us all' (the 'all' refers to the members of the Committee of Vice-Chancellors and Principals). He was appreciated as highly by his colleagues in the Arts and other faculties as by those in his own profession, but this is not the place

(even if I were competent to do so) to detail his numerous imaginative and important contributions to the progress and expansion of his University. Suffice it to say that he guided it with signal skill through the difficult years of the Second World War and during the post-war period of rapid development when it doubled in size within three years. His administration was marked by wisdom, honesty, tact and sympathy, yet when the occasion demanded he could be firm and even brusque, but he was always fair, and he enjoyed the affection and respect of his colleagues to a degree that must have been almost unique.

He combined his duties as Vice-Chancellor and Prof. of Anatomy until 1938 when he resigned from his Chair and was succeeded by Frederick Wood Jones. Thereafter he was appointed to a personal Chair in Experimental Neurology; he was prevented by the pressure of administrative duties from continuing his teaching and research, a situation he always regretted, but he always placed duty above pleasure and he felt his responsibility to the University as a whole took precedence over his personal desires. In 1956 when he retired he was made an Emeritus Professor.

His ability as a scientist was recognized by his election to the Fellowship of the Royal Society in 1927 and he was the first Manchester medical graduate to achieve this high distinction. Under the inspiration of Elliot Smith his research interests were predominantly neurological and usually they had a distinct applied flavour. He collaborated successfully with several of his surgical friends and colleagues, notably Sir Harry Platt, Sir Geoffrey Jefferson and Profs. John Morley and E. D. Telford, in the elucidation of various problems. Throughout his academic career, and especially during and after the First World War, he frequently visited the hospital wards and in consequence he was elected Honorary Advisory Anatomist to the Manchester Royal Infirmary. Stopford's research work can be divided roughly into three phases—his investigations on the blood supply of the brain-stem and some other parts of the brain, his anatomical and clinical studies on sensation based mainly on the examination of many patients with periperhal nerve injuries (a large number resulting from war wounds), and his explorations of the structure and functions of various parts of the autonomic nervous system. He published over sixty papers and a monograph on 'Sensation and the Sensory Pathways' (1930), besides contributing sections to several text-books. A bibliography of his original publications is available in the *Manchester University Medical School Gazette* (1956, **35**, no. 3, pp. 108–109). As Vice-Chancellor he was unable to indulge his flair for correlating basic and clinical research, but had he continued his anatomical career he would undoubtedly have enhanced his already high reputation as a scientist.

His worth was recognized both nationally and locally and one can mention only the more important of his many appointments other than those mentioned above. At various times he was a member of the University Grants Committee and was once Chairman-elect of this body (illness caused his resignation before he could assume office), he was a member of the General Medical Council and Chairman of its Business Committee, Chairman of the Universities Bureau of the British Empire, Vice-Chairman of the Trustees of the Nuffield Foundation, Vice-Chairman of the Nuffield Hospital Fund, Vice-Chairman of the Interdepartmental Committee on Medical Schools, Deputy Chairman of the Committee of Vice-Chancellors and

Principals, Vice-President of the Anatomical Society (he was also one of our Life Members), a member of the Medical Advisory Committee of the University Grants Committee, a member of the Ministry of Health Advisory Committee on Distinction Awards for Consultants, a member of the Home Office Advisory Committee on the Administration of the Cruelty to Animals Act, a member of the Council of the Royal College of Physicians, a member of the British Elective Committee of the Commonwealth Fund, a member of the Committee on Higher Agricultural Education, first Chairman of the Manchester Regional Hospital Board, Chairman of the Manchester Royal College of Music, Chairman of the John Rylands Library, a member of the Board of Governors of the United Manchester (Teaching) Hospitals, a member of the Board of Governors of Manchester Grammar School, a member of the Manchester College of Technology Sub-Committee of the Manchester Education Committee and Chairman of the Manchester, Salford and Stretford Joint Hospital Board. This last-mentioned Board, which Stopford was instrumental in forming to co-ordinate the activities of the local hospitals, later assumed considerable importance because the success of this venture influenced those who planned hospital organization under the National Health Service. His experience in this post doubtless explained his outstanding success as first Chairman of the Manchester Regional Hospital Board.

Prof. Stopford was the recipient of many honours. He was awarded honorary degrees by the Universities of Dublin (Sc.D., 1937), Leeds (D.Sc., 1939), Cambridge (Sc.D., 1951), Manchester (LL.D., 1951; M.A., 1957), Liverpool (LL.D., 1953) and Durham (D.C.L., 1957). He was elected F.R.S. in 1927 and was granted Honorary Fellowships of the Royal College of Physicians in 1942 and of the Royal College of Surgeons in 1955. He was knighted in 1941, created a K.B.E. in 1955, he became a Freeman of Manchester in 1956 and his name was included in the first list of life peers in 1958.

He was a stimulating and lucid lecturer who helped to rescue anatomy from the topographical morass into which it had fallen and he did much to narrow the gap which had developed between the basic medical sciences themselves and also between them and clinical practice. He began using radiological methods to teach living anatomy in 1919 and he was the first anatomist in this country to take over from physiology the full responsibility for teaching histology. To the end of his life he retained an interest in everything appertaining to education and his knowledge of teaching and research in all their manifold aspects was utilized, as the abbreviated list of his appointments indicates, by many governmental and other bodies. It is not generally recognized that he was the chief inspiration for many of the far-sighted reforms in medical education recommended in the 'Goodenough Report'.

With so many commitments Stopford had little leisure time, but he was never too busy to meet and discuss problems with members of his staff and students. He had an easy amiability which made him equally at home with the young and the old, the obscure and the famous, and he was a frequent and welcome participator in the informal discussions and arguments in the university common room. Latterly he had to contend with several serious illnesses, yet such was his sense of duty that he largely ignored medical advice to curtail his activities. He did resign from some of his many honorary appointments, but he carried an immense administrative load

until the day he retired. He was a keen gardener and on Saturdays he sometimes relaxed by visiting the university sports fields or by attending a football match. As a young man he was an ardent soccer player and he was a lifelong supporter of the Manchester City and Manchester United teams. He was an influential member of the Presbyterian Church of England and officiated at many functions on its behalf.

He married Lily Allan, another Manchester medical honours graduate, in 1916 and she and one son survive him. Theirs is the greatest loss; but we who served under him revere the memory of a distinguished anatomist and a supreme administrator and marvel that one who had gained so many honours could remain so human, unaffected and kind. Manchester University was singularly fortunate in having three outstanding men —Elliot Smith, Stopford and Wood Jones—in succession as its Professors of Anatomy, and while Elliot Smith and Wood Jones attained greater reputations in the scientific field, neither equalled Stopford in his versatility or in his accomplishments. We have lost one of the great anatomists of this century.

G. A. G. MITCHELL

MICHAEL KENT WRIGHT, M.B., Ch.B., B.Sc., B.Sc. (Hons. Anat.), M.Sc.

On 14 January 1961, Dr Michael Kent Wright died at Manchester.

Dr Wright was born of British parents in East Africa in 1923. He came to South Africa at the age of 7 years and, after an excellent school career, matriculated from Hilton College, Natal. He entered the Witwatersrand Medical School in 1940, having been refused enlistment in the armed forces on account of health. During the course of his medical studies he took a Medical B.Sc., a B.Sc. Honours and then a Master of Science degree, graduating as a Bachelor of Medicine and Bachelor of Surgery at the end of 1947. Whilst a clinical student he served as a graduate demonstrator and as a lecturer in the Department of Anatomy.

After qualifying, he had a wide experience, serving as a Lecturer in Anatomy from 1948 to 1950 and as Senior Lecturer until 1953. Thereafter, he filled the positions of Neuro-Physiologist to the Department of Applied Electrophysiology at the National Hospital for Nervous Diseases, Queen Square, London (1953-54); Registrar at Queen Square (1954); Medical Officer to the Neuro-Surgery and Neurology Departments of the Johannesburg General Hospital, as well as Assistant Neurologist at Tara Hospital, Johannesburg. At the beginning of 1959 he resumed his position as Senior Lecturer in Anatomy at the University of the Witwatersrand, which he held until, in August 1960, he was appointed Lecturer in Experimental Neurology at the University of Manchester.

Dr Wright's special field of interest was the nervous system. He had published some twenty-five works, ranging in subject matter from the pre-optic region of the mammalian brain to the electro-encephalographic correlates of consciousness. His text-book, *Fibre Systems of the Brain and Spinal Cord*, was first published in 1952 by the Witwatersrand University Press; it has since run to a second edition (1959). The presentation of the text illustrates Dr Wright's lucidity of expression, which made him an outstanding and engaging teacher.

Dr Wright showed himself to be a calm and efficient organizer and a very hard worker. He was a determined individual of noteworthy ability and competence. He felt deeply on his subject and never tired of stressing the need for a more rigorous approach in modern electro-encephalography. Of sensitive nature, he was quick to express himself when he felt that his views or his subject were being called into question.

Mike Wright's passing has shocked his many friends and colleagues, all of whom will wish to extend their deepest sympathy to his relatives in South Africa and to his wife and children at their family home in North Wales.

P. V. TOBIAS

DOUGLAS ERITH DERRY, M.C., M.B., Ch.B., Hon. F.R.C.S.

Prof. Derry died on 20 February 1961 at his home in Essex. He was 87 years of age.

For over 30 years he was Professor of Anatomy in the University of Cairo (Kasr-el-Aini medical school). He was appointed in 1919. This post gave him many opportunities for archaeological and anthropological observations in the Nile valley. This region was not new to him for in his early post-graduate years he had been attached to the archaeological survey of Nubia as an anatomist. Perhaps the best known fact about Prof. Derry is that he was the first anatomist to examine the mummy of the Pharoah Tutankhamun.

He had an affection for the Nile valley and had planned to continue living in his Cairo flat after his retirement. But the political upheaval in Egypt at the end of 1951 necessitated his departure early in 1952 as it did that of all holders of British passports attached to Egyptian universities. During his tenure of the Cairo chair he was an outstanding figure in Egypt and acted as host to most of the distinguished scientific visitors from this country.

As Professor of Anatomy he was a patient and painstaking teacher. As an examiner he was very systematic and thorough and no student would have dared to 'coax' him to change his verdict. To a colleague meeting him at the end of his career the general impression was that of his courage and precision. The burden of a chair in a medical school admitting over a thousand students a year can be imagined. One of his pupils, Prof. El-Batrawi, succeeded him in the Kasr-el-Aini chair, and that is what he would have wished. Another of his pupils, Prof. Aasar, became the first Professor of Anatomy in the University of Alexandria, and yet another, Prof. Shafik Abd-el-Malek, filled the anatomy chair at the Abassia medical school in Cairo.

All through the First World War Derry served in the Royal Army Medical Corps. He was awarded the Military Cross. Before the outbreak of war he had been working at University College, London as lecturer in Physical Anthropology in the Anatomy Department when Prof. Thane held the chair. During this period he worked also in Prof. Karl Pearson's department (Biometrics) where he carried out extensive researches on the bones of the human skeleton in conjunction with members of Karl Pearson's staff. The results of vast numbers of measurements were used in sexing the bones found in one of the London plague pits of the 1665 outbreak. Karl Pearson always remained a firm friend.

It is to be regretted that Derry published no autobiographical account of his years in Egypt. When Francis Bacon in his *New Atlantis* urged that travelling scholars should go from these islands to distant lands carrying stores of knowledge and technical skills from this country, he also advocated their return with stores of experience from the countries they had visited to be shared with their fellow scientists here. Much of such experience is not suitable for publication in scientific journals and so is lost to succeeding generations.

U. FIELDING

REVIEWS

Primatologia, Vol. 4, Lieferung 5. Edited by H. Hofer, A. H. Schultz and D. Starck. (Pp. vi+66 pp.; 28 figures; S.Fr. 20.) Basel: S. Karger. 1961.

This small Lieferung devoted to the vertebral column and thorax of the Primates is from the pen of Prof. A. H. Schultz who has spent a lifetime in the detailed study of the Primate skeleton and its growth.

Besides collating the principal results of his own studies, the author has surveyed the wide literature of the subject and has presented an excellent synthesis of the phylogenetic and ontogenetic changes in the vertebral column and thoracic skeleton of the Primates, including the frequency of anomalous conditions, the shifts in pelvic attachment and the variations in relative proportions. Illustrations are well up to the author's established standards and include some new figures.

W. C. OSMAN HILL

Advances in the Biology of Skin. Volume I. *Cutaneous Innervation.* Edited by W. Montagna. (Pp. xii+203; 118 illustrations; 63*s.*) Pergamon Press Ltd. 1960.

This book is based on the proceedings of the Brown University Symposium on the Biology of Skin held in 1959 under the chairmanship of Dr W. Montagna. It covers the following topics presented by twelve contributors: neurohistology of the skin; distribution of cholinesterase in the cutaneous tissues; relation of nerve fibre size to modality of sensation; central pathways of afferent impulses from the skin; mechanism of common sensibility; sensation of itch under biological and pathological conditions.

The nine chapters give a balanced account of the various aspects of cutaneous innervation in light of recent advances. More consideration could be given to the cytology of the end organs. No mention is made of the ultrastructure of the receptors, but this is not surprising since very few investigations have been carried out in this field. The volume is generously illustrated and well edited and provided with a useful subject-index. The book can be recommended to anatomists as well as to physiologists and clinicians for its concise style and as a valuable source of reference.

N. CAUNA

Nerve Endings in Normal and Pathologic Skin. By R. K. Winkelmann. (Pp. viii+195; 42 illustrations; 60*s.*) Springfield, Illinois, U.S.A.: Charles C. Thomas. 1960.

One cannot feel anything but sympathy for an author who embarks upon the hazardous journey through this topic somewhat reminiscent of Christian's progress. The skill and adroitness with which the author weaves his way through this thorny subject only serves to replace sympathy with admiration. Here is a well illustrated succinct account of cutaneous nerve endings drawn from the author's experience and that of others who have risked their reputations studying this intriguing problem. This book provides interesting and stimulating reading helping to clarify one's viewpoint instead of adding to the existing confusion. Much of this confusion has been due to the temptation to romance about the possible function of these organs and the clarity of this book can be ascribed to restraint exercised by the author when considering functional aspects. The order in which the receptors are described and the emphasis this places upon their importance may be a source of disagreement with some readers but this is, after all, the author's personal viewpoint which we are all, fortunately, still permitted to possess and cherish. Perhaps a more critical review of the work of others would have increased the interest of the book and is a weakness

comparable to the tendency of the young to provide, with little or no discussion, a tabulated list of the contributors to knowledge of a subject in their essays and other writings. This one criticism does not, however, prevent my wholehearted recommendation of a valuable little book. W. HEWITT

The Nervous System. By G. M. WYBURN. (Pp. vii + 184; 108 figures; £1. 15*s*.) London: Academic Press. 1960.

This book presents a new approach to the nervous system both because of the arrangement of the material and because structure and function are considered together. In the preface the author states that he hopes the book will serve as an introduction to neurology for medical students, as a starting-point for post-graduates and as a text for medical auxiliaries. There is no doubt that within these terms of reference he has succeeded admirably; readers who forget these modest aims may criticize unjustifiably parts of the book as being rather superficial. The basic structure and function of the neuron, including electron microscopic findings, are considered in the first chapter. The main sensory systems are then treated in turn, and in each of them the course of the impulses is traced from the periphery to the different terminations in the central nervous system. Other chapters deal with the motoneurons, speech mechanisms, the hypothalamus and the autonomic nervous system. The chapters on the brain-stem reticular formation, the cerebral hemispheres and consciousness, and chemical regulation in the central nervous system are particularly valuable as little has been written on these subjects outside specialised monographs and reviews. The bibliographies given at the end of each chapter are up to date and include the major references. In the description of the neuron the term cell-body or soma would be preferable to the cell (which is equivalent to the whole neuron), the diagrams of the action potential of a nerve fibre and of the compound action potential would be more meaningful if time intervals were given on the abscissae, and in some of the discussion of more recent work certain statements are made more dogmatically than is warranted. These, and similar criticisms which can be made, are relatively minor ones in a book which has already been found useful for medical students beginning their course. The book could also be recommended to colleagues whose main interests are not neurological as it discusses, briefly and clearly, the major advances of the past two decades. T. P. S. POWELL

Electron Microscopy in Anatomy. Proceedings of a Symposium held by the Anatomical Society of Great Britain on the Ultrastructure of Cells. Edited by J. D. BOYD, F. R. JOHNSON and J. D. LEVER. (Pp. iii + 288; 50*s*.) London: Edward Arnold. 1961.

This volume contains the published proceedings of a Symposium on 'The Ultrastructure of Cells' held by the Anatomical Society of Great Britain and Ireland in April 1959, in the Department of Anatomy, University College London, and all the papers in it are concerned with electron microscopy. The holding of such a symposium was an important sign of the times, and it is heartening to read good solid papers on this subject illustrated by excellent micrographs from a considerable number of Departments of Anatomy (and allied studies) in this country.

The papers in this Symposium may be divided into three categories. At the beginning are a relatively small number of short papers describing current instrumental and specimen techniques; the paper by A. W. Agar on 'Present and Future Possibilities of Electron Microscopy' provides an excellent short introduction to the instrumental aspects of the subject for newcomers—and others. The papers on specimen techniques are less comprehensive.

The main body of papers in the volume include a number which fall into the 'short review'—in some cases mainly of the author's own work—category; others describe more restricted studies and resemble closely those original papers which one would find in a

journal devoted to ultrastructure. In the former category are to be found papers by J. D. Robertson on 'The Unit Membrane'; by G. E. Palade on 'The Secretory process of the pancreatic exocrine cell'; and by J. D. Lever on 'Fine structural appearance in relation to function in certain secretory organs'. Not all of these will be unfamiliar to the diligent reader of the current scientific literature, but their prescence here undoubtedly adds greatly to the value of the volume as a general guide to the kind of anatomical work which is being done by this technique.

Those which fall more into the 'original paper' category comprise a further twelve papers which deal with, amongst other things (to name a few at random), the ultrastructure of synapses of the cerebral cortex, membrane interrelationships during meiosis, the secretory products of ameloblasts, and the attachment of myofibrils to the sarcolemma at the muscle-tendon junction. The common feature of these papers, and of practically all the other ones in this volume is their very high general scientific standard, a state of affairs which is by no means automatically achieved when a powerful, impressive and complicated new instrument comes into general use. Symposia of this kind should go far towards maintaining good standards in the subject, particularly if they can be published more promptly than this one.

H. E. HUXLEY

Quantitative cellular haematology. By J. M. YOFFEY. (Pp. xv+122; 10 tables and 17 figures; 44*s*.) Oxford: Blackwell Scientific Publications. 1960.

Prof. Yoffey's book is one of a series of monographs edited by Prof. Bethell of Michigan under the title of *American Lectures in Hematology*, another contributor from Britain being Rosemary Biggs of Oxford. The author's life-long interest in the lymphocyte is well known, and in this book he sets forth the results of the experiments he has carried out with his colleagues on guinea-pig lymphocytes which concern their genesis, transport and destinations in the body. As the title indicates, he has paid attention principally to quantitative techniques including ones recently evolved, such as the use of tritium labelled thymidine, in order to support information he has accumulated on morphological grounds. He also refers to the work of other authors in this field in an extensive bibliography, and, for both these reasons, this attractively produced monograph would be read with profit by all those interested in the lymphocyte.

Prof. Yoffey is a staunch disciple of Maximow in that he considers the lymphocyte to be a stem cell which can give rise to both erythrocytes and granulocytes, and he argues that the evidence he has accumulated supports him in this view. Some of the results he has obtained with the guinea-pig certainly show that much is yet to be learned about the fate of the lymphocyte, but the vital evidence needed to prove his thesis is still missing, namely, the observation of the metamorphosis of the living cell, and its cinematographic recording. Certain of his statements are unacceptable to one brought up in the field of human pathology. For instance myeloid metaplasia in the human lymph node is clearly a feature of the sinusoidal region and not of the germinal centre. Again, it is hard to accept the principle that the production of antibodies in response to a new stimulus is a form of specialization which can only occur in a primitive and undifferentiated cell.

However, although there is likely to be unrepentant opposition to some of Prof. Yoffey's ideas, there is no question that the results of his experiments and his interpretations command respect in a field which is at the present time one of the advancing fronts in biological research.

J. L. PINNIGER

Man's Posture: Electromyographic studies. By J. JOSEPH. (Pp. x + 88; 23 illustrations; 44*s*). Springfield, Illinois: Thomas. 1960.

The electromyographic studies reported in this short but important monograph, one of the American Lecture Series in Orthopaedic Surgery, help to clarify the relative importance of muscles and ligaments in the stabilization of joints. Many of the chapters are devoted to

a consideration of the muscles in use when a person 'stands at ease'. In this particular posture (defined in the text), exceptionally sensitive instruments can detect no electrical activity in much of the lower limb musculature and only limited activity in the erector spinae. The hip and knee joints are stabilized by the appropriate ligaments and, possibly, the psoas major. Regular swaying movements of the body relieve these ligaments from prolonged uninterrupted tension.

More startling is Dr Joseph's conclusion that living muscles can relax completely. In his opinion—and the evidence presented is certainly convincing—the concept that muscles at rest exhibit 'tone' in the sense of a maintained contractile tension due to intermittent activity in scattered motor units should be abandoned. It is not clear how this conclusion can be reconciled with modern neurological opinion concerning the role of muscle spindles, but Dr Joseph emphasizes that the notion of balanced 'tone' in opposing muscles is based upon a misunderstanding of Sherrington's statements.

This book is a pleasure to read, not only because of the ordered marshalling of evidence and argument but also because of its lucid style and excellent illustrations. It will be essential reading for all who interest themselves in the mechanics of the body and the functions of muscles.

C. H. BARNETT

BOOKS RECEIVED

Cerebral Angiography in the Rabbit. By P. G. Jeppson and Tord Olin. Lunds Universitets Arsskrift. N.F. Avd. 2, Bd. 56, Nr. 14. 1960. (Pp. 3–56, 35 figs., kr. 8.50). Lund: C. W. K. Gleerup.

Atlas der gynäkologischen Anatomie. By Heinrich Martius and Käthe Dryosen, 1960. (Pp. v+118, 134 figs. DM. 49.) Stuttgart: Georg Thieme Verlag.

Essentials of Human Embryology. By Frank D. Allen, 1960. (Pp. vii+225, 120 figs. 40*s*. New York and London: Oxford University Press.

The Growth of Scientific Physiology. By G. J. Goodfield, 1960. (Pp. 11+174. 18*s*. London: Hutchinson.

Abstracts of Human Developmental Biology, vol. 1, no. 1. (Abstracts nos. 1–164). Edited by G. ten Cate, 1961. (Pp. 1–50: Subscription rate £6. 18*s*. per annum.) London: Excerpta Medica Foundation.

Slice Reconstructions of Human Cerebral Sections. By Wendell J. S. Krieg, 1960. (Pp. 643–670, $ 1.) Evanston, Illinois: Brain Books.

Die Anwendung von Enzymen und chemischen Agentien in der histochemischen Methodik. Edited by T. H. Schiebler. *Acta Histochemica*, Suppl. Bd. II, 1961. (Pp. v+270, D.M. 64.40.) Jena: Gustav Fischer Verlag.

Foetal and Neonatal Physiology. Edited by K. W. Cross. *British Medical Bulletin*, vol. 17, no. 2, 1961. (Pp. 79–176, £1.) British Council, London.

PROCEEDINGS OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

FEBRUARY 1961

An Ordinary Meeting of the Society for the Session 1960–61 was held on Friday, 24 February 1961, in the New Hall of the London Hospital Medical College, London, E. 1.

The President (Prof. J. D. Boyd) and Prof. Ruth E. M. Bowden occupied the Chair at the various Sessions.

The following are the authors' abstracts of papers presented.

The electron microscopy of mucus secreting cells. By B. A. Young and F. R. Johnson. *London Hospital Medical College*

In the present investigation mucus secreting cells in the stomach, small intestine and large intestine of several species have been investigated with the electron microscope. Particular attention has been paid to (1) the free and contact surfaces of the cells, (2) the problem of resting cells, (3) the mechanism of discharge, and (4) the mechanism of elaboration of secretion.

The findings indicate that the Golgi apparatus is involved in the formation of the secretion which in its progress towards the apex of the cell remains enclosed in smooth membranes. These membranes are responsible for the mucus appearing in a granular form and they are discharged with the mucus into the intestinal lumen. Mucus first accumulates in the 'empty' cell in a central position and as it increases in amount it displaces the endoplasmic reticulum with its associated granules laterally. Discharge does not occur until the cell has become distended with secretory granules at which time only the apical position of the accumulated mucus is lost; granules can always be seen in proximity to the Golgi apparatus. These appearances suggest that although the discharge of mucus may occur periodically the elaboration of secretion is probably a continuous process.

Observations on the synthesis of ferritin in subcutaneous macrophages. By A. R. Muir and L. Golberg. *University of Edinburgh and Benger Laboratories Ltd.*

A small proportion of a dose of iron-dextran, injected into the subcutaneous tissue of the mouse abdominal wall, remains at the site of injection and is ingested by local macrophages, which within 24 hr. contain large numbers of Prussian Blue-positive, electron-dense granules.

Excess iron in the cytoplasm is normally stored as ferric hydroxide micelles in the protein molecule ferritin. As iron-dextran has a macromolecular structure which is distinguishable in electron micrographs from the characteristic images produced by ferritin, it is possible, in this material, to observe the raw material and the end product of a synthetic process. When ferritin first appears in the macrophage, it is uniformly distributed throughout the cytoplasm as individual molecules, and there is no special concentration around the ingestion vacuoles. A high concentration of cytoplasmic ferritin is accompanied by a few molecules in the nucleoplasm, but usually none is seen in the internal matrix of mitochondria. Less than 8 days after an injection, some cells contain aggregations of ferritin surrounded by a membrane (siderosomes), as well as isolated molecules in their cytoplasm.

These observations suggest that ferritin synthesis is not associated with any of the cytoplasmic organelles. The administration of iron stimulates the synthesis of an iron-free protein, apoferritin, which is invisible in the electron microscope, and it is suggested that this apparent synthesis of ferritin is merely the incorporation of iron into preformed apoferritin molecules scattered throughout the cytoplasm.

Application of the scanning electron-probe X-ray microanalyser to mineralized tissues. By A. Boyde, V. R. Switsur and R. W. Fearnhead. *London Hospital Medical College, and Cavendish Laboratory, University of Cambridge*

The Scanning Electron-probe X-ray Microanalyser utilizes the property of X-ray emission which results from the bombardment of elements by an electron beam. The instrument provides qualitative and quantitative data which can be related to the topography of the surface of the specimen. Thus it is possible to identify an element and to obtain an accurate estimate of the quantity of this element in the surface of the sample. The present instrument is capable of identifying elements having atomic numbers greater than $Z = 12$.

Polished cut surfaces and sections of human and rodent teeth were examined using this instrument. In rodent incisors a clear identification of iron in the enamel can be obtained. In human dentine differences in the quantity of calcium present in different regions can be demonstrated. Quantitative assessment of elements in biological material provides, however, special problems of interpretation. The experimental evaluation of these problems was discussed.

Wound contraction in the guinea-pig. By C. A. C. Charlton, D. I. R. Higton, D. W. James, A. R. Nicol and J. O. Stewart. *University College, London*

The diminution in area of an excised skin wound during healing is termed wound contraction; it may be accelerated by splinting a wound for 10 days to minimize movement of the skin edges and then removing the splint. In previous works conflicting results have been reported for the rat and rabbit on the one hand, and the guinea-pig on the other. The present experiments were undertaken to determine whether these differences in the contraction mechanism were species specific.

A 2×2 cm. square of skin was excised from each side of the thorax in thirty-three guinea-pigs. The wounds were splinted to minimize contraction, and 10 days later a 1×1cm. square area was tattooed on their granulation tissue. These squares were freed from their surroundings by incisions down to the wound bed on the 10th or 12th day with the splints undisturbed, or on the 12th after desplinting on the 10th. In all cases incision led to contraction of the central island and of the granulation tissue peripheral to it, and in wounds biopsied without splints to retraction of the wound margins.

It was concluded that wound contraction in the guinea-pig, as in the rat and rabbit, is mediated by the granulation tissue itself.

Use of hyaluronidase, prior to fixation, in a method for the histochemical demonstration of cholinesterase. By Terry Williams. *University College, Cardiff*

Using the method of Koelle & Friedenwald, modified by Coupland & Holmes (*Quart. J. micr. Sci.* (1957), 98), on a great variety of tissues, patchy or incomplete staining was often observed. Such preparations were unsuitable for histochemical assessment or photography. It seemed likely that these shortcomings might be due, in part at least, to uneven fixation or uneven penetration of the fixative.

In an attempt to correct or reduce these technical defects, tissues were infiltrated with hyaluronidase before death or immediately after excision, and afterwards fixed and processed in the usual way. It was found that improvement in reliability of the technique was achieved with some tissues but not with others. Tissues or organs which with the ordinary method showed greatest patchiness, seemed to benefit most from the hyaluronidase treatment.

The faviol reactions in histology. By M. A. MacConaill and E. Gurr. *University College, Cork*

Violamine 3B is an acid xanthene dye. Used with acid fuchsin as is light green it forms three compounds (faviolic acids) analogous to the falgic acids previously described and demonstrated, even in their reaction to orange G with and without glucose. The faviolic technique is often better than the falgic for the clear demonstration of moderately and weakly erythrophile elements by violet and blue colours, respectively.

A useful modification of the faviolic technique is to include Sun Yellow G in the violamine solution; this changes the colour of the weakly erythrophile elements from blue to yellow and brightens the red of the strongly erythrophilic elements. It is a very clear stain for chromosomes. In addition, this modification increases the birefringence of already birefringent elements and also increases the contrast of stained elements viewed by phase contrast.

Observations on the structure of elephant ivory. By A. E. W. Miles and A. Boyde. *London Hospital Medical College*

Surfaces of elephant ivory cut transverse to the axis of the tusk show a regular pattern composed of two intercrossing systems of radiating curved lines which begin at the centre of the tusk and sweep outwards in smooth curves to the periphery. Analysis shows that the pattern consists essentially of an alternation of light and dark areas rather like a chequer-board but, because the pupal surface is curved, the diagonals of the 'chequer-board' are also curved.

Longitudinal sections cut radially to the centre of the tusk show that the tubules pass in regular sinuous curves across the thickness of the ivory. Tangential sections show that the tubules are arranged in groups or segmental columns within which the curvatures of the tubules are all in the same phase but are in the reverse phase from those of the adjacent columns. The surface of ivory appears dark or light according to whether the rays of light strike convexities or concavities of the groups of curved tubules. It is concluded, therefore, that the pattern seen on the cut surface of elephant ivory is a manifestation of a particular arrangement of its tubular system.

Specializations in the pinniped placenta. By R. J. Harrison and B. A. Young. *London Hospital Medical College*

Certain specializations in the placenta of three species of Pinnipedia, which may be associated with physiological aspects of diving, have already been described by the authors (*Verh. Anat. Gesell.* Zurich, 1959). Further material from *P. vitulina* and *H. grypus* has become available. The often incompletely annular chorioallantoic placenta is characterized by large maternal sinusoids lined with irregular thickened endothelium lacking the lace-like appearance described by Dempsey and Wislocki in the cat. They are surrounded by a thick, dense membrane containing reticulum against which the trophoblastic cell membranes are frequently folded. The endothelium of foetal capillaries is thin and the perivascular membrane is thinner than the maternal layer and appears laminated. The placental membrane is reduced in places to less than $1{\cdot}0\,\mu$ in thickness of which over 50 % may consist of perivascular substance. The tall columnar cells of the marginal 'haematomata' possess branched microvilli, phagocytosed aggregates and ingested maternal red blood cells. The haematomata contain anisotropic bilirubin crystals and cholesterol esters. It is suggested that these appearances indicate a mechanism for transferring iron to a foetus which is known to have a higher blood volume, greater red cell count and larger red cells than most mammals. The columnar cells of the paraplacental region are arranged in rosettes opposite the mouths of glands and their apposed surface possesses microvilli.

An experimental study of the avian visual system. By W. M. Cowan, L. Adamson and T. P. S. Powell. *University of Oxford*

The central projection of the retina has been studied in the pigeon using a variety of silver degeneration techniques. Within 5 days of eye-enucleation the optic nerve is severely degenerated and the degenerating fibres appear to decussate completely in the optic chiasma to enter the ventral part of the contralateral optic tract. From the optic tract this degeneration can be traced into the following thalamic nuclei: nucleus lateralis anterior, the so-called lateral geniculate nucleus, and the nuclei externus and superficialis synencephali. More posteriorly fibres pass from the marginal optic tract to the outer layers of the optic tectum and to the tectal grey. Coarse degenerating fibres leave the dorsum of the optic chiasma to form the basal optic root; this can be followed along the ventral aspect of the diencephalon to its termination in the ectomamillary nucleus. Severe cellular degeneration in the isthmo-optic nucleus and the unusual nature and time source of the fibre degeneration in the axillary or isthmo-optic tract are suggestive of retrograde degeneration in a centrifugal system which is in agreement with the findings of earlier authors. An appreciable number of normal fibres persist in the stratum opticum of the tectum and can be followed through the marginal optic tract to the region of the optic chiasma; their precise origin and termination are, however, unknown. It is of interest in view of physiological work that no degeneration has been found in the hypothalamus or in those thalamic nuclei which are known to project upon the telencephalon.

Further observations on long ascending tracts in the reptilian spinal cord. By L. R. Robinson. *St Mary's Hospital Medical School, and University of Otago*

In a previous communication (November 1960) evidence was presented for the existence of a typical fasciculus gracilis in the spinal cord of *Lacerta viridis*, derived from dorsal roots entering the hind-limb enlargement. Further work has demonstrated the existence also of a fasciculus cuneatus derived predominantly from dorsal roots cranial to the 9th, and ending in the medulla cranial and lateral to the fasciculus gracilis. Some evidence was found for direct connexions from dorsal root fibres to the anterior horn and also for a small proportion of descending fibres in the dorsal columns.

The ascending degenerating fibres previously demonstrated in the superficial part of the lateral columns have now been traced in preparations made by the Holmes silver method and by a modified Nauta technique (Guillery, Shirra and Webster) into the brain stem. Here they occupy a superficial position giving off collaterals and possibly terminal branches to the reticular formation of the hind brain. Some can be traced ventral to the 5th nerve to curve dorso-medially into the cerebellum on the same and on the opposite side. The crossing occurs close to the decussation of the 4th nerve. Degenerating fibres were identified in the granular layer of the cerebellum. A smaller number of degenerating fibres could be traced into the midbrain. It appears therefore that in the superficial part of the lateral columns of the spinal cord there are many spino-cerebellar and spino-reticular fibres, and possibly some spino-mesencephalic fibres.

The counting of human sweat glands. By J. S. Weiner. *University of Oxford*

A major difficulty in accepting the available figures for the total number and regional density of human eccrine sweat glands is the remarkably small area of the body surface which different authors have sampled, ranging from as little as 1 to not more than about 12 cm.^{2}. No one seems to have ascertained the minimum area necessary to give a reliable total estimate.

In this study the body has been divided into eight regions and counts made by the plastic impression method on fields of 1 cm.^{2}. In the first instance, sweating in each field was provoked by intra-dermal injections of 20γ mecholyl on two subjects. In each region a

succession of fields was counted until an estimate for the region as a whole, stable to within particular confidence limits (as judged by Barnard's sequential test) was obtained. A simpler procedure involves stimulation of the glands by exposing the subject for 1½ hr. to an air temperature of 32° C., combined with immersion of one leg in water at 42–43° C. From results on five subjects it would seem that about 90 cm.^{2} should be counted to obtain a representative estimate of the total.

The effect of diet on the oxidation sensitivity of rat haemoglobin. By W. K. Metcalf. *University of Bristol*

The increased sensitivity to oxidation of the haemoglobin of children, pregnant women and patients with carcinomata is paralleled by a similar change in sensitivity in young and pregnant rats. As a working hypothesis, it was assumed that the increased sensitivity was due to the demands on the metabolism of rapidly growing tissue, and that the changes in the red cells were due to deprivation of a normal blood constituent.

Starvation of adult rats caused a marked increase in the sensitivity to oxidation within 24 hr. and by 48 hr. their blood was as sensitive as that of pregnant or rapidly growing young rats. Feeding quickly restored the blood to normal levels of sensitivity.

The various constituents of their normal diet were then fed to, or injected into, starving rats. Only components of the rat cubes containing the Vitamin B complex caused any perceptible return of the blood to normal. When individual members of the B complex were administered separately, only riboflavine gave a positive response.

Pregnant and young rats, whose blood showed the usual increased sensitivity to oxidation were injected with riboflavine. Within a few hours of the injection oxidation sensitivity was within normal limits.

Comparison of the different teratogenic effects of two commercial samples of trypan blue. By F. Beck. *University College, Cardiff*

Recently (Beck, Spencer, & Baxter, *Nature, Lond.* (1960), **187**), it was shown that commercial samples of trypan blue could be purified by recrystallization so that the product did not produce as many foetal resorptions when injected into 8½-day pregnant rats as did the parent compound.

Two commercial samples of trypan blue are compared in this communication. One marketed by Flatters & Garnett is highly teratogenic and produces a high resorption rate when injected into Wistar rats at 8½ days of pregnancy, the other marketed by G. T. Gurr is non-teratogenic and produces a low resorption rate when an equal amount of azo dye (by titration) is injected on a weight for weight basis at the same stage of pregnancy. The LD 50 for rats has been calculated for both samples and is compared with their teratogenic effects.

Some examples of the type of external malformations produced by the potent sample of the dye were shown.

Aminopterin and the explanted chick embryo. By D. S. O'Dell and J. McKenzie. *University of Aberdeen*

After 21 hr. incubation, chick embryos were explanted by the New technique and then incubated over an albumen medium for a further 21 hr. The development of embryos treated with from 3×10^{-11} g. to 3×10^{-6} g. aminopterin has been compared with that of the controls. At the higher doses, brain, blood channel, heart and somite formation were all inhibited. The most persistent effect with minimal doses was the absence of blood channels. Inhibition of growth may be produced by either ventral or dorsal administration of the drug.

The effects of aminopterin could be overcome by the simultaneous administration of

5×10^{-4} g. DNA Na salt. The same amount of thymidine or of RNA Na salt gave no such protection when administered separately, but a 1:3 mixture of the two gave some protection.

Twenty-one hours after the addition of the drug, there was no evidence of necrosis or significant alteration of mitotic activity.

The significance of these findings was discussed.

Venous modifications in two rare marine mammals. By J. D. W. Tomlinson and R. J. Harrison. *London Hospital Medical College*

Specimens of the Laysan Monk Seal (*Monachus schauinslandi*) from Hawaii and of the Cruciger Dolphin (*Lagenorhynchus cruciger*), killed south of the Cape of Good Hope, have been made available by the British Museum (Natural History). Both species are represented by only very few individuals, neither has been dissected before. Nothing is known of their diving habits.

The Laysan Seal was immature and displayed coarctation of the aorta. The posterior vena cava was duplicated as in *Phoca* but possessed a complicated arrangement of anastomotic channels between the two limbs not hitherto described in mammals. The common trunk of the vena cava was enlarged in its intrahepatic portion and a spherical hepatic sinus was present. An incomplete striated muscle caval sphincter was separated from the diaphragm by a band of connective tissue. An azygos vein and an extradural vertebral vein were present. There were no pericardial retia.

The Cruciger Dolphin, unlike most cetaceans, possessed only a single posterior vena cava. There was no hepatic sinus, but a partial sphincter and a muscular 'sling' from the diaphragm were present. Two large intercommunicating extradural intravertebral veins lay ventro-lateral to the spinal cord in the thoracic region: retial tissue also surrounded the cord. The intravertebral veins drained by large vessels emerging between the third to sixth thoracic vertebrae into a longitudinal vein lying on the neck of the ribs. Marked intrathoracic and pericardial masses of retial tissue were present.

The vascular modifications found in these two marine mammals was compared with those previously described in other Pinnipedia and Cetacea by the authors and by Slijper (*Capita zool.* 1936).

A case of persistence of the primitive olfactory artery. By D. B. Moffat. *University College, Cardiff*

This anomaly occurred in a 71-year-old male who died of a carcinoma of the stomach. The left anterior cerebral artery gave off a small branch which passed forwards and ventrally and pierced the dura of the anterior cranial fossa and, after giving off a few small dural branches, divided into three vessels which pierced the cribiform plate of the ethmoid. It supplied the upper part of the nasal cavity and its largest branch ran downwards and forwards on the nasal septum almost to the floor of the nose. The artery is believed to represent a persistent primitive olfactory artery, which in the embryo is a large vessel formed by the termination of the cranial ramus of the internal carotid artery.

Observations on the innervation of chick skeletal muscle fibres. By B. Mackay and B. L. Ginsborg. *University of Edinburgh*

Neuromuscular junctions on fibres from various skeletal muscles of the chick have been demonstrated by staining for sites of cholinesterase concentration. Some muscle fibres possess many neuromuscular junctions positioned at intervals along the fibre, while other fibres have one or perhaps a few junctions. In many of the muscles studied there are both varieties of fibre, but in the anterior latissimus dorsi only fibres with many neuromuscular junctions, and in the posterior latissimus dorsi only fibres with few junctions.

The significance of these results was discussed.

Pinocytosis and dense cytoplasmic granules in the cultured fibroblast.

By A. F. HAYWARD. *University of Glasgow*

The ultrastructure of cultured fibroblasts (strain L) and that of their irradiation-induced giant cells, has been investigated by the electron microscopy of thin sections after the incorporation of 'Thorotrast' (Testagar) into the medium.

The substance is first adsorbed on to the plasma membrane, it is subsequently found on the inner surface of small superficial vacuoles and then free within the larger deeper vacuoles. In the same micrographs it occurs in the matrix of dense cytoplasmic granules. The latter, approximately 0·3 mμ across, have a single outer membrane and some have granules or lamellar arcuate structures within the matrix. The lamellae may be arranged parallel to the outer membrane and the granules with a double outer membrane may be explained in this way. The possible relationship to mitochondria was discussed. In the case of irradiation giant cells, the same sequence is observed but is more rapid in execution and the 'Thorotrast' is incorporated into the dense granules at an earlier stage. This may perhaps be regarded in conjunction with the increased volume of these cells and their possible need to obtain nutrients by a means other than diffusion. The giant cells do not divide and, particularly in the older ones studied, the number of dense granules is greater than in the normal cells grown under the same conditions.

Application of infra-red spectroscopy to mineral systems with special reference to mammalian enamel.

By J. C. ELLIOTT. *London Hospital Medical College*

The work of Posner and Duyckaerts (*Experientia* (1954), **10**) on the infra-red spectra of teeth has been repeated and extended.

The infra-red spectra of a number of carbonate compounds have been studied in the region 4000–700 cm.$^{-1}$. This shows that the 877 cm.$^{-1}$ absorption peak of the CO_3^{2-} group in calcite depends on its immediate co-ordination by calcium and is different in aragonite ($CaCO_3$) and other synthetic carbonates. The position of the peak is found to be at about 877 cm.$^{-1}$ in a mineral apatite and elephant enamel. The carbonate peak at about 1500 cm^{-1} is a doublet. This finding contrasts with that in respect of calcite in which the corresponding peak is single, suggesting that the samples studied contain no or very little calcite.

The positions of other absorption peaks have been determined, some of which have been identified.

The blood supply to the enamel organ of the rodent incisor.

By D. ADAMS. *University of Edinburgh*

Reith (*Anat. Rec.* (1959), **133**) showed that the so-called papillae of the enamel organ in the rat incisor were really a series of transverse folds in the external enamel epithelium. This finding has been confirmed and extended to other rodents, and the relationship of the blood vessels to this series of folds has been investigated. Large capillaries, supplied by long slender arterioles running in the long axis of the tooth, lie in the troughs between these folds, forming a plexus external to the outer enamel epithelium. Venous drainage of this plexus is to a network of wide channels lying against the bony wall of the tooth socket. The connexion between the venous network and the capillaries is along the cement enamel junction.

Variations in the density of the capillary plexus occur along the length of the tooth, being greatest in the stellate reticulum region and least where the enamel organ atrophies. The findings do not support the concept that the minerals required for maturation come from pulp vessels.

To meet the needs of continuous eruption the arterial branches to the capillaries either grow in length, or migrate along the plexus. A third possibility is that the ameloblasts move forward relative to the capillary plexus.

The innervation of tooth germs in man. By R. W. FEARNHEAD.
London Hospital Medical College

The pattern of the innervation of human tooth germs during various stages of development has been studied in thirteen foetuses and seven children. Some of the specimens were fixed in Bouin and some in 10 % neutral formol-saline. Serial sections were cut at 10μ and two of the specimens stained with haematoxylin and eosin. The remainder were impregnated with silver, either by the De Castro method or by a modified Holmes's technique.

The tooth bud develops in an already innervated mesenchymal environment. Nerve fibres become associated with the epithelial component of the tooth germ in the earliest stages of tooth development. The outermost layer of the enamel organ, which is richly vascularized, retains a remarkably rich innervation during the period of amelogenesis. The dentine papilla on the other hand only receives the 'pioneer' fibres of its nerve supply when the tooth germ has reached the 'bell' stage of development.

THE SUBPLACENTA OF THE GUINEA-PIG: DEVELOPMENT, HISTOLOGY AND HISTOCHEMISTRY

By J. DAVIES, E. W. DEMPSEY and E. C. AMOROSO*

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The subplacenta of the guinea-pig, a specialized zone of the chorion between the placental disc and the basal decidua, was first accurately described by Duval whose monograph (1892) contains a critical review of the early literature. His recognition of the origin of the placental syncytium from the foetal ectoderm and of the inversion of the yolk sac, which laid the foundation of our present knowledge of the rodent placenta, also enabled him to trace the origin of the subplacenta from the chorionic ectoderm. Lacking this essential basis of facts, previous writers (Bischoff, 1866; Ercolani, 1877; Creighton, 1878; Laulanié, 1886) attributed the origin of the subplacenta to the uterine or decidual tissues. Duval observed that the subplacenta occupied the fundus of the central mesenchymal core of the placenta and so called it the 'roof of the central excavation'. The term 'subplacenta' was first used by Minot (1889) in connexion with the rabbit placenta and was later applied to the guinea-pig. The vascularization of the subplacenta exclusively by foetal vessels was observed by Tafani (1886) and Wislocki (1921).

Mossman (1937) stated that the accessory placenta or subplacenta was definitely known to exist in only two animals, the guinea-pig and the porcupine, both members of the Hystricomorph group of rodents. It has been shown recently (Perrotta, 1959) that it is found in other members of this group (e.g. coypu, chinchilla, agouti, viscacha).

The structure of the subplacenta from the time of its appearance about the 16th day to its involution before term has been studied by histological and histochemical means. Theoretical evidence for the function of the subplacenta either as a centre of gonodatrophic activity or as a site of absorption of proteins from the decidua is presented.

MATERIALS AND METHODS

Pregnant guinea-pigs were obtained from dealers or were bred in the laboratory for the critical early stages. The stage of gestation, using the weight and crown-rump length of the embryos or fetuses as criteria, was assessed by reference to the table of Needham (1931). The uterine swellings in the early stages were fixed intact. In the later stages they were opened and the placentae removed. Fixation for general histological purposes was in Bouin's fluid followed by sectioning in paraffin and staining with haematoxylin and eosin or iron haematoxylin without a counterstain. The periodic acid-Schiff (PAS) method was carried out on similar sections with and without treatment with saliva before staining. Alkaline phosphatase activity, using

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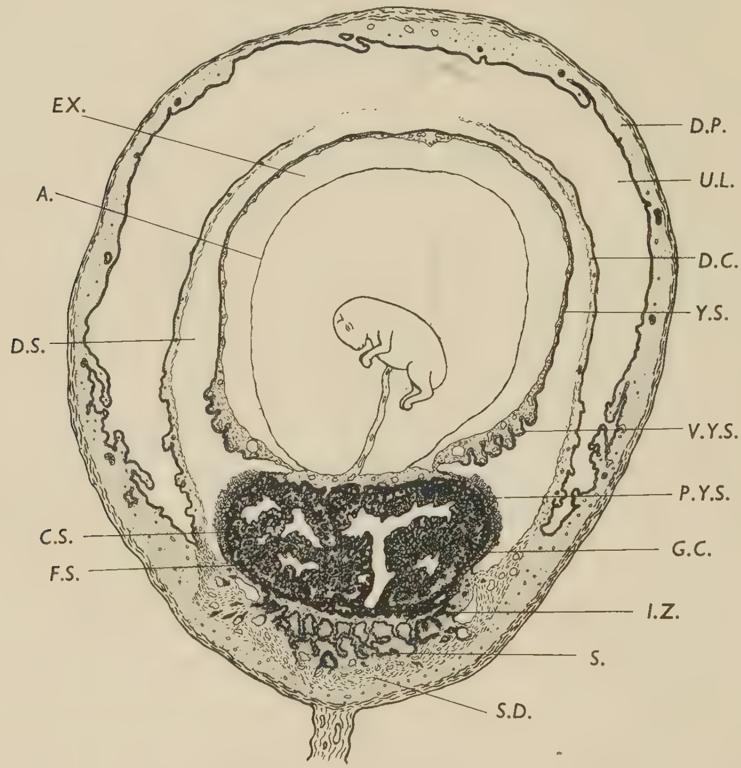
sodium glycerophosphate as a substrate, was studied in paraffin sections after fixation in cold alcohol according to the usual technique. Frozen sections after formalin fixation were stained with Sudan black for the identification of lipids. Cytoplasmic basophilia was observed after fixation in Zenker-formol and staining with 1 % toluidine blue: the sections were examined in water.



Text-fig. 1. Diagrammatic cross-section of the guinea-pig uterus about the middle of pregnancy (35 days). *A.* amnion; *C.S.* coarse syncytium; *D.C.* decidua capsularis; *D.P.* decidua parietalis; *D.S.* decidual cavity; *EX.* exocoelom; *F.S.* fine syncytium; *G.C.* chorionic giant cells; *I.Z.* intermediate zone; *P.Y.S.* parietal wall of yolk sac; *S.* subplacenta (cytotrophoblast in solid black, syncytium stippled); *S.D.* subplacental decidua; *U.L.* uterine lumen; *V.Y.S.* villous part of visceral yolk sac; *Y.S.* visceral wall of yolk sac.

RESULTS

The foetal membranes at mid-gestation

The arrangement of the foetal membranes at about the 35th day of gestation is illustrated in Text-fig. 1. The placenta is mesometrial. The dedicua capsularis breaks down at about the 30th day, allowing the decidual cavity and the uterine lumen to communicate. The chorio-allantoic placenta is made up of two types of syncytium: a coarse type containing maternal blood sinuses but no foetal vessels and a fine type containing both maternal sinuses and foetal vessels. The yolk sac is inverted and

consists of a visceral and a parietal wall. The visceral wall is made up of a smooth and a villous portion; its columnar epithelium faces towards the decidual cavity and later towards the uterine lumen. The parietal wall of the yolk sac is incomplete and is applied to the bulging convexity of the placental disc, fading out at the chorio-decidual angle. Deep to the parietal endoderm is a layer of chorionic giant cells which merge on their deep surface with the marginal syncytium of the chorio-allantoic placenta.

The subplacenta consists of folded lamellae of cytotrophoblast which give rise to syncytial trophoblast on their decidual surface. Between the subplacenta and the chorio-allantoic placenta is a plane of foetal mesenchyme which passes between the cytotrophoblastic lamellae of the subplacenta and carries vessels to them. The subplacenta merges peripherally with the marginal syncytium of the chorio-allantoic placenta through an intermediate or transitional zone. A zone of necrotic decidua lies deep to the subplacenta: through it pass the maternal vessels supplying the chorio-allantoic placenta. These vessels pierce the subplacenta at its margin, and at an early stage show a replacement of their endothelial walls by invasive trophoblastic elements from the subplacenta. A thin layer of normal decidua intervenes between the necrotic zone and the myometrium. The plane of cleavage of the placenta at parturition takes place between this layer and the myometrium.

The subplacenta separates easily from the placental disc due to the plane of mesenchyme between them. It separates with more difficulty from the decidua but then appears as a bulging ovoid structure of firm consistency and grey in colour, in contrast to the redness of the chorio-allantoic placenta. It appears laminated on its foetal surface and smooth and gelatinous in consistency on its decidual surface from which a mucus-like material may be expressed.

The implantation site at the 15*th day*

The most conspicuous feature of the implantation site at the 15th day (Pl. 1, fig. 1) is the deeply basophilic cytotrophoblastic layer which appears in sections as a V with slightly sinuous walls. The hollow of the V, the 'central excavation of Duval', contains a small amount of cellular mesenchyme in which there is no evidence of angioblastic activity. The cytotrophoblast is multilaminar (Pl. 1, fig. 2) and mitotic figures are common. External to it is a thick spongy layer of syncytial trophoblast which is derived by proliferation from the cytotrophoblast. Its meshes are filled with maternal blood. The syncytial cytoplasm is granular and basophilic though less so than that of the cytotrophoblast.

The marginal syncytium is highly invasive, especially in the region of the floor of the central excavation. The invasive outgrowths from this area are hollow for the most part and contain maternal blood (Pl. 1, fig. 2); they penetrate the basal decidua to about one-third of its depth. The syncytial outgrowths penetrate the decidua as multinucleated masses along the outer wall of the parallel decidual capillaries (Pl. 1, fig. 3), as described by Creighton (1878). The endothelial wall of each capillary then disappears, presumably as a result of the cytolytic action of the syncytial trophoblast, and the maternal blood is liberated into a trophoblastic 'tube', the lumen of which is larger than that of the original capillary (Pl. 1, fig. 4). The trophoblastic tubes may be traced into continuity with the large maternal sinuses adjacent

to the cytotrophoblastic layer and are the forerunners of the major placental vessels which later pierce the subplacenta.

A further specialization of the marginal syncytium is the formation of chorionic giant cells (Pl. 1, fig. 2). Outside the giant cell layer is the columnar epithelium of the parietal wall of the yolk sac which projects freely into the decidual cavity (Pl. 1, fig. 2). The hypertrophied appearance of these endodermal cells suggests that they may be involved in absorption of materials from the decidual cavity.

In summary, at the 15th day, there is no evidence of the local differentiation of the subplacenta from the general chorio-allantoic mass. Its subsequent appearance in the floor of the central excavation is foreshadowed by the concentration of invasive activity in the trophoblast of this area.

The implantation site at the 16*th day*

The wall of the central excavation shows two well-defined zones (Pl. 2, fig. 5): an upper of coarse syncytium characteristic of the chorio-allantoic placenta and a lower representing the subplacenta. The cytotrophoblast of the chorio-allantoic zone is reduced in thickness and the layer of coarse syncytium derived from it is correspondingly thicker. There are relatively few invasive syncytial outgrowths from this region and the giant cells and parietal endoderm form a more or less uninterrupted layer. The subplacenta begins about half-way down the sloping wall of the central excavation and is distinguished by three main features: (1) the thickness and folding of the cytotrophoblastic layer, (2) the vacuolation of the syncytium, and (3) the large number of invasive outgrowths. The decidua is now penetrated by the syncytial trophoblast to about a half of its depth. The subplacental syncytium becomes vacuolated immediately after its origin from the cytotrophoblast (Pl. 2, fig. 6). The vacuoles or lacunae are bounded by attenuated septa of syncytial cytoplasm and contain small acidophilic droplets and an occasional maternal red blood cell. Small islands of syncytial cytoplasm with their associated nuclei are scattered throughout the vacuolated syncytium in the angles between groups of lacunae. The giant cells and parietal endoderm are conspicuous at the edge of the subplacental area, but form a discontinuous layer due to the passage of many invasive syncytial outgrowths across the decidual cavity. The giant cells arise by differentiation from the marginal syncytium (Pl. 2, fig. 6). The nucleus is large with coarse chromatin masses: the cytoplasm consists of a basophilic perinuclear zone and a peripheral acidophilic zone in which large droplets may frequently be observed.

The droplets within the subplacental syncytium are strongly PAS positive (Pl. 2, fig. 7), and since the reaction is unaltered by previous treatment with saliva they are probably glycoprotein or mucoprotein in nature. Other acidophilic droplets are PAS negative and may represent fragmented maternal erythrocytes. The transition zone between the subplacenta and the chorio-allantoic placenta is shown in more detail in Pl. 3, fig. 8. The syncytium is solid and thickened between the two areas, and the subplacental lacunae bordering the transitional zone are smaller and more irregular than in the rest of the subplacenta: they also contain more acidophilic droplets, some of which are strongly PAS positive and others are negative.

PAS positive, saliva resistant material is also found in the following sites (Pl. 2, fig. 7 and Pl. 3, fig. 8): as large irregular masses in the chorionic giant cells, as small

droplets in the apical cytoplasm of the cells of the visceral wall of the yolk sac, and in some of the decidual cells beyond the limit of trophoblastic invasion. A feature of the decidual cells in this area is the beaded PAS positive capsule surrounding individual cells. In the zone of invasion (Pl. 3, fig. 9) the decidua is necrotic, and large amounts of amorphous acidophilic (and PAS positive) material are liberated into the decidual cavity, presumably under the cytolytic influence of the trophoblast. The cells of the parietal wall of the yolk sac show a PAS positive brush border, but the cytoplasm contains no droplets.

The appearance of a decidual vessel in the necrotic zone is illustrated in Pl. 3, fig. 10. There is no endothelium and the wall of the vessel is composed of trophoblastic cells containing scattered PAS positive droplets. It is limited externally by a PAS positive basement membrane. The wall of such a vessel has been termed 'endotrophoblastic' since its syncytial or cytotrophoblastic nature is undecided.

Glycogen is present in small amounts in the cytotrophoblast of the subplacenta and in considerable amounts in the subplacental syncytium. It is absent in detectable amounts from the chorio-allantoic placenta. Glycogen is also present in the parietal endoderm and the chorionic giant cells. The decidual cells contain very little glycogen in contrast to those of the rabbit.

The implantation site at the 17th and 18th days

Pl. 4, fig. 11 shows the increased bulk of the lateral placental masses and the conspicuous subplacental zone occupying the fundus of the central excavation. The latter is wider than in previous stages and contains foetal vessels in various stages of development.

The chorio-allantoic placenta shows no added features except for the increased complexity of the maternal blood channels. The increase in thickness of this part of the placenta takes place by proliferation of syncytium from a marginal layer of cytotrophoblast on the foetal surface of the placental disc. Medial to the attachment of the visceral wall of the yolk sac the marginal placental epithelium is entirely cytotrophoblastic. Lateral to its attachment there is a layer of chorionic giant cells on the deep surface of the parietal endoderm: these cells appear to differentiate from the marginal cytotrophoblast which disappears from this area at an early stage.

The cytotrophoblastic layer of the subplacenta is thicker and more folded than at the 16th day and is in contact on its foetal surface with the angioblastic mesenchyme of the central excavation. The invasive activity of the placenta is now restricted almost entirely to the subplacental zone and the decidua is penetrated almost to its full depth (Pl. 4, fig. 11). There is a conspicuous decidual cavity deep to the subplacenta which is partly filled with acidophilic debris derived from the breakdown of the basal decidua and is crossed by invasive tongues of syncytium from the region of the subplacenta (Pl. 4, fig. 12). The terminal ramifications of these syncytial outgrowths are arrested at the edge of a zone of normal decidua between the necrotic zone and the myometrium. Here they form a strongly basophilic 'junctional zone' of syncytial giant cells (Pl. 4, fig. 11).

The implantation site from 18 to 25 days

This is the 'période d'achèvement du placenta' of Duval (1892) during which the vascular mesenchyme of the central excavation invades the chorio-allantoic placenta forming a series of foetal lobules of fine syncytium. The residual syncytium at the periphery of the placental disc and between the lobules is not invaded by foetal mesenchyme and forms the coarse syncytium. A third type of syncytium is found in the subplacenta: it is avascular and is characterized by its vacuolation and invasive activities.

The continued folding of the cytotrophoblast and the extreme vacuolation of the subplacental syncytium during this period are illustrated in Pl. 4, fig. 12 and Pl. 5, fig. 13. The thickness and proliferative activity of the cytotrophoblast are at their maximum at the end of this phase and the large syncytial vacuoles contain many acidophilic PAS positive droplets (Pl. 5, fig. 14). The invasion of the decidua by syncytial outgrowths of the subplacenta is on the wane by the 25th day and many of the invasive outgrowths which are present in the earlier stages may subsequently degenerate. Those which persist are associated with the major placental vessels which enter and leave the chorio-allantoic placenta by piercing the margin of the subplacenta. As they pass through the subplacenta the walls of these vessels are composed solely of syncytial trophoblast which is not vacuolated in the immediate vicinity of the lumen of the vessel but merges peripherally with typical vacuolated syncytium (Pl. 5, fig. 14). At no stage are cytotrophoblast and maternal blood found in contact.

The subplacenta from the 25th to the 45th day

The subplacenta shows a progressive increase in thickness and an increase in the complexity of the cytotrophoblastic lamellae (Pl. 6, fig. 15). The syncytium is also more compact in the region of the cytotrophoblastic layer, but becomes more vacuolated in the direction of the decidua and also at the margin of the subplacenta. The intermediate or transitional zone, where the subplacental syncytium merges with the marginal syncytium of the chorio-allantoic placenta is greatly modified by the condensation and fusion of the syncytial nuclei, forming irregular intensely basophilic masses (Pl. 6, fig. 15).

The production of syncytium from the cytotrophoblast is at its maximum during this period and results in a progressive thinning of the cytotrophoblastic layer which is reduced in many areas to a single layer of cells. The syncytium is also compact in contrast to its coarsely vacuolated appearance in the earlier stages. The pattern of droplet formation also alters and presents difficulties of interpretation which have not been fully resolved. The compact syncytium, which has been derived most recently from the cytotrophoblast, shows a delicate cytoplasmic stippling of PAS positive droplets which appears in low powers of the microscope as a faint general PAS positive tinge. As the syncytium is traced away from the cytotrophoblastic layer it becomes more vacuolated and contains droplets of larger size. The vacuoles then appear to run together, forming a lattice-like pattern of PAS positive droplets (Pl. 6, fig. 16). This pattern may arise by a confluence of individual vacuoles, as described above, or alternatively may reflect the manner of development of the syncytium from the parent cytotrophoblast. The droplets are probably extracellular,

as suggested by electron microscopic findings (Davies, Dempsey & Amoroso, 1961), in which case the confluent system of droplets may demarcate developmental units of syncytium derived from a single cytotrophoblast or group of cytotrophoblasts. A study of this problem may give some insight into the vexing problem of the formation of syncytium from the cytotrophoblast (see Wislocki & Bennett, 1943), and the subplacenta of the guinea-pig seems to be an ideal site for its future investigation.

By the use of the purple dye aldehyde-fuchsin (Gomori, 1950) it has been possible to differentiate the PAS positive elements of the subplacenta and related parts of the placenta. After Bouin fixation the PAS positive droplets of the subplacental syncytium are strongly aldehyde-fuchsin positive (Pl. 11, fig. 29). The stippled syncytial cytoplasm close to the cytotrophoblast stains more lightly with the aldehyde-fuchsin but presents a definite purple tinge. The cytotrophoblast, which is PAS negative, also shows a faint purple coloration with the dye. Other PAS positive elements of the placenta, e.g. the intracellular droplets of the chorionic giant cells, the droplets of the visceral wall of the yolk sac, the basement membranes of the vessels, the amorphous masses in the necrotic zone of the decidua, the uterine milk in the decidual cavity, and the sporadic cytoplasmic droplets of the decidual cells fail to stain with aldehyde-fuchsin. Previous oxidation of the sections with sulphuric acid and potassium permanganate (Wilson, 1952) converts all these aldehyde negative elements into positive ones, the stain then resembling closely the PAS reaction.

Glycogen continues to be abundant in the subplacental syncytium but is absent from the cytotrophoblast. Other sites of glycogen deposition are as described at the 16th day. The subplacental glycogen is no exception to the general observation that placental glycogen is well preserved by Bouin fixation (Davies, 1956). Its appearance in droplet form is, however, probably artifactual since it is diffusely distributed in the cytoplasm after alcohol fixation and in the electron microscope (Davies *et al.* 1961).

Lipid is absent from all elements of the subplacenta, as shown by staining of frozen sections with Sudan black and after fixation in Dalton's osmium-dichromate fixative. Small amounts are seen, however, in the syncytial extensions of the subplacenta into the decidua and in the walls of the maternal vessels. The chorio-allantoic syncytium, the normal and necrotic decidual cells and the endoderm of the visceral wall of the yolk sac contain considerable lipid. It is absent from the endoderm of the parietal wall of the yolk sac and the chorionic giant cells.

Alkaline phosphatase activity is absent from the subplacenta, though marked in the fine syncytium of the chorio-allantoic placenta, thus confirming the observations of Wislocki, Dempsey & Deane (1946). Cytoplasmic basophilia is conspicuous in the cytotrophoblast of the subplacenta and slight in the syncytial trophoblast. Basophilic material is also found as a perinuclear zone in the chorionic giant cells (Pl. 2, fig. 6) but is absent from the associated layer of parietal endoderm.

Decidual vessels

A large placental vessel in the basal decidua is shown in Pl. 7, fig. 17. Its endotrophoblastic wall is spongy and the interstices are filled with maternal blood. It is limited externally by a PAS positive basement membrane. Solid extensions of the

endotrophoblastic wall pass deeply into the decidual tissues, each of which is surrounded by a basement membrane. These endotrophoblastic roots extend almost to the myometrium and come into intimate relationship with the modified decidual cells of the junctional zone. The trophoblast bordering the lumen of the vessel is not vacuolated; peripherally, however, it is vacuolated and resembles the subplacental syncytium with which it is continuous. The vacuoles contain many acidophilic and PAS positive droplets (Pl. 7, figs. 18, 19). The structure of the walls of the maternal vessels in their course through the subplacenta is essentially the same. As they enter the chorio-allantoic placenta the endotrophoblast merges with the coarse syncytium and the PAS positive droplets disappear (Pl. 8, fig. 20).

Junctional zone

The junctional zone between the necrotic and the residual zone of normal decidua is illustrated in Pl. 8, fig. 21. The necrotic zone is occupied by a 'magma' (Duval) of amorphous material and cellular debris which is acidophilic and also PAS positive. The terminal extensions of the endotrophoblastic roots are vacuolated, the result either of the removal of glycogen during the preparation of the sections or of degenerative changes. Trophoblastic giant cells are also found in the junctional zone and are intimately mingled with giant cells of decidual origin (Pl. 8, fig. 21). The trophoblastic giant cells have many small nuclei of even size and a finely granular basophilic cytoplasm which contains a variable amount of glycogen but no other PAS positive material. The decidual giant cells are distinguished from the trophoblastic giant cells by the pleomorphism of their nuclei, their 'ground-glass' cytoplasm which has no particular affinity for acid or basic dyes, and the characteristic capsule of PAS positive material which surrounds individual cells (Pl. 8, fig. 21). The decidual giant cells are clearly derived from the hypertrophy of normal decidual cells and there are no indications of mitotic activity or of fusion of cells. Both the normal and giant decidual cells contain small amounts of glycogen and other PAS positive elements which are saliva-resistant; these cytoplasmic materials are released into the necrotic zone of the decidua following the disintegration of the giant cells. The junctional zone of the guinea-pig placenta shows many interesting similarities to the basal plate of the human placenta (see Wislocki, 1951).

Chorionic giant cells and parietal endoderm

The chorionic giant cells and the parietal endoderm are well developed from the 20th to the 45th day (Pl. 9, fig. 22). The parietal endoderm is a pseudo-stratified columnar epithelium resting on a basement membrane which separates it from the layer of chorionic giant cells. The intercellular spaces of the parietal endoderm may be greatly dilated, accounting for the tufted or villous appearance of the epithelium (Pl. 9, fig. 23). Even at the same stage of gestation, however, the cells of the parietal endoderm may be compact with no significant dilation of the intercellular spaces (Pl. 9, fig. 24). Irregular extensions of PAS positive material from the basement membrane pass into the intercellular spaces, sometimes reaching the luminal edge of the cells where they are prevented from entering the decidual cavity by a system of terminal bars.

The subplacenta from the 45th day to term

After the 45th day the waning activity of the cytotrophoblast is indicated by the scarcity of mitotic figures and by its reduction to a single layer (Pl. 10, fig. 25). In many areas the cytotrophoblast disappears so that the syncytium rests directly on the basement membrane. The mesenchymal septa between the cytotrophoblastic lamellae are also less cellular and more fibrous, and contain fewer vessels. The syncytium degenerates and there is a progressive accumulation of acidophilic and PAS positive material between the residual islands of syncytial cytoplasm. The characteristic pattern of droplets within the syncytium is lost but may persist as late as the 60th day (Pl. 10, fig. 26). The extent of degeneration of the subplacenta in the latter part of pregnancy is very variable. At term it still shows evidence of its original histological organization, the cytotrophoblastic layer being indicated by discontinuous rows of pycnotic nuclei enclosing islands of acidophilic and PAS positive material (Pl. 11, fig. 27). The endotrophoblastic walls of the maternal vessels are well maintained at term and show little sign of degeneration apart from the deposition of fibrin-like material along their inner wall adjoining the lumen. They continue to show conspicuous amounts of PAS positive material in droplet form.

The parietal endoderm and the layer of chorionic giant cells degenerate after about the 45th day. They are recognizable in the last week of pregnancy on the bulging surface of the placental disc over a short area lateral to the attachment of the visceral wall of the yolk sac. At term the two layers have disappeared and are replaced by a layer of cellular connective tissue which fades out in relation to the chorio-decidual angle.

DISCUSSION

The subplacenta, using the terminology of Minot (1889), is essentially a modification of the strip of chorion which connects the two lateral masses or cotyledons of the placenta and occupies the fundus of the central excavation. In the rabbit this area remains relatively simple up to about the 13th day: it is mainly cytotrophoblastic but gives rise to a small amount of abortive syncytium and some free chorionic giant cells which pass into the uterine milk at the site of implantation (Pl. 11, fig. 28). After this time the area is apparently converted into typical chorio-allantoic syncytium with no distinguishing features (Duval, 1890). No subplacenta, in the sense in which this term is used in the guinea-pig, is now considered to exist in rodents except the Hystricomorphs (Mossman, 1937; Perrotta, 1959). Nevertheless, it seems valid to consider the subplacenta in these forms as a modification of the strip of chorion which unites the lateral placental masses in other rodents and which, in the latter, becomes merged into the general chorio-allantoic syncytium. Duval (1892) accurately described the invasiveness of the syncytium in the guinea-pig, beginning about the 11th day and continuing throughout the period of vascularization of the foetal placenta from the 18th to the 25th day. He also described the compact layer (cytotrophoblast), the plasmodium (syncytium) and the layer of chorionic or ectodermal giant cells in relation to the parietal wall of the inverted yolk sac. His description of the vacuolation of the syncytium failed to include the characteristic droplets within the syncytial lacunae. Speaking of the significance of the sub-

placenta, Duval writes: 'le toit de l'excavation centrale nous parait n'avoir aucune importance ni au point de vue morphologique, ni au point de vue fonctionnel.'

The salient features of the subplacenta are its emergence from the general chorio-allantoic mass about the 16th day, the persistence of the cytotrophoblast until a late stage in gestation, the character of the syncytium and the presence of periodic acid-Schiff droplets within its lacunar spaces. A further important feature is the invasiveness of the subplacental syncytium and its extension into the basal decidua, which results in the firm attachment of the placenta to the maternal tissues and in the replacement of the walls of the decidual (placental) vessels by trophoblastic cells. The endotrophoblastic wall of these vessels also becomes vacuolated and accumulates a large amount of PAS positive material.

The presence of large amounts of PAS positive material within the syncytium, tentatively considered as glycoprotein or mucoprotein in nature, suggests two possible functions of the subplacenta: (1) that it is a centre of gonadotrophic activity, and (2) that it is a specialized chorionic area involved in the absorption of materials from the decidua. In favour of the hormonal activity of the subplacenta are the following facts.

(1) The known gonadotrophins of the pituitary and of the human and mare placenta are glycoproteins (Pearse, 1953) and stain with the PAS method. It has been shown in this paper that the PAS positive droplets of the subplacenta also stain with the aldehyde-fuchsin method of Gomori. The histochemical significance of this stain is unknown and has been described in a wide variety of normal mammalian tissues by Halmi & Davies (1953). Pertinent observations from this survey include the positive staining of the gonodatrophic cells of the anterior pituitary (beta cells) and that of the intracellular droplets of the cytotrophoblastic cells in the basal plate of the human placenta. The latter have been thought to be implicated in the production of human chorionic gonadotrophic hormone (Wislocki, 1951). It is not known if the PAS positive material of the endometrial cups in the mare stains with aldehyde-fuchsin. No other elements of the guinea-pig placenta give a reaction with this stain which appear, in this site at least, to be quite specific for the subplacental droplets.

(2) The PAS positive droplets appear in the subplacenta at the time of its development from the general chorio-allantoic mass, at about the 16th day. This is a critical period in the gestation of the guinea-pig since the oestrous ovulation is suppressed. The suppression is assumed to be due to the maintenance of luteal activity, the result of some luteotrophic influence emanating from the implantation site. Rowlands & Short (1959) have shown that the progesterone content of the corpus luteum increases two or three times in the pregnant guinea-pig from about the 11th to the 23rd day. According to these authors this rise may coincide with the production of a luteotrophin either from the placenta or from some other source.

(3) The subplacenta continues to flourish throughout the first two-thirds of gestation, showing a continuous formation of syncytium from the cytotrophoblast up to at least the 50th day, and in some instances considerably later. The syncytium immediately becomes vacuolated and PAS positive material appears within it, first as minute droplets within the cytoplasm, and later as larger droplets within the lacunar spaces. These events may be reasonably construed as secretory in nature.

(4) The cytotrophoblast ends its proliferative activity about the 50th day and the syncytium slowly degenerates. This involution of the placenta in the last weeks of pregnancy may well be correlated with the waning activity of the corpus luteum which is known to persist throughout gestation and to show histological signs of degeneration within a few weeks of term (Loeb, 1911). A disturbing feature of the arguments for the luteotrophic function of the subplacenta are the observations of some (see Courrier, 1945) that the ovaries of the guinea-pig can be removed early in pregnancy without interrupting it, presumably because extraovarian progesterone is available (e.g. from the placenta or the adrenal). Deanesley (1960) has confirmed earlier observations that implantation can occur in the guinea-pig after ovariectomy: the pregnancy is not maintained, however. The maintenance of the supply of progesterone from extraovarian sites by gonadotrophins of placental origin is a possibility. Thus, when chorionic gonadotrophin is added to the fluid perfusing the human placenta *in vitro* there is an increase in the output of steroid substances from labelled precursors (Pincus, 1956).

A clue to the gonadotrophic function of the subplacenta may lie in the fact that this peculiar organ is found only in the Hystricomorph group of rodents (see above). All of these rodents have long gestation periods (porcupine, 121 days; chinchilla, 111 days; coypu, 135–150 days; agouti, 104 days; viscacha, 3 months; guinea-pig, 58–72 days (Asdell, 1946; Eckstein & Zuckerman, 1956) and in two of these forms, the North American porcupine (Mossman & Judas, 1949) and the viscacha (Pearson, 1949) accessory corpora lutea are known to be formed during pregnancy. This situation resembles that in the mare, the giraffe and the elephant in which accessory corpora lutea appear and may be correlated with the existence of placental gonadotrophins (Amoroso, 1955). The formation of accessory corporea lutea in pregnancy has not been documented in the human female nor in the guinea-pig. If the gonadotrophic function of the guinea-pig subplacenta is substantiated, it is of interest that this is an example of gonadotrophin production by the syncytial trophoblast, in contrast with the human female where the gonadotrophin is produced by the cytotrophoblast (Wislocki & Bennett, 1943) and in the mare by the decidual tissues of the endometrial cups (see Amoroso, 1952).

Favourable arguments can be made on theoretical grounds for the function of the subplacenta in the guinea-pig as a mechanism for the absorption of materials from the decidua, especially those of high molecular weight such as proteins or polysaccharides. The early invasion of the decidua by the syncytium and the conversion of this tissue to a necrotic mass of 'uterine milk' containing much PAS positive material suggest that such material may be absorbed by the syncytium and carried to the subplacenta by streaming movements within the cytoplasm. The chorionic giant cells, which limit the placental syncytium externally and contain large amounts of PAS positive material, have been shown to have phagocytic properties (Wislocki, 1921). The hypertrophied columnar cells of the parietal yolk sac with their dilated intercellular spaces also suggest that they may represent an important site of transfer of materials to or from the decidual cavity.

The speculations on the possible function of the subplacenta underline the limitations of the morphological method where the direction of movement of materials across cell boundaries is to be determined. Insight into its function may be gained

by experimental investigation, particularly if extended to other members of the Hystricomorph rodents and correlated with ovarian and general reproductive physiology. Perrotta (1959) has suggested that the main significance of the subplacenta is as a germinal centre for the continued production of new syncytium by which the placenta increases in bulk with advancing gestation. No evidence has been found to support this view. Indeed, the separation of the cytotrophoblastic layer of the placenta from the adjoining chorio-allantoic mass by a distinctive layer of vacuolated syncytium (Pl. 2, fig. 7 and Pl. 4, fig. 12) seems to preclude this possibility. All the proliferative activity of the subplacental cytotrophoblast is directed to the production of highly vacuolated and invasive syncytium, and this proliferation, moreover, is in the direction of the decidua and away from the chorio-allantoic placenta. A study of Perrotta's photographs indicates that the subplacenta of the Canadian porcupine (*Erithizon dorsatum*) differs from that of the guinea-pig in the greater folding and complexity of the cytotrophoblastic lamellae with perhaps a reduction in the amount of intervening syncytium.

A further physiological peculiarity which distinguishes the guinea-pig subplacenta is its inability to concentrate protein bound radioactive iron (^{55}Fe) injected intravenously into the mother in which feature it differs strikingly from the chorio-allantoic placenta (Stewart, 1961).

Electron microscopic observations on the subplacenta and related parts are presented in another paper (Davies *et al.* 1961) and have resulted in a considerable clarification of many of the morphological and histogenetic problems raised in this paper.

SUMMARY

1. The subplacenta of the guinea-pig arises about the 16th day of pregnancy as a specialization of the chorion in the floor of the mesenchymal core or 'central excavation' of the placenta.

2. It consists from the time of its first appearance and in subsequent stages of a folded layer of cytotrophoblast which is in contact with foetal mesenchyme on its foetal surface and gives rise to syncytial trophoblast on its maternal surface.

3. The proliferative activity of the cytotrophoblastic layer and the new-formation of syncytium are at their maximum from about the 18th to the 25th day. After this time the cytotrophoblast becomes reduced in thickness and the syncytium becomes more compact.

4. The subplacenta begins to degenerate after about the 50th day and is necrotic at term.

5. The salient histochemical feature of the subplacenta is the presence of large amounts of periodic acid-Schiff (PAS) positive material within the lacunae of the syncytial trophoblast. This material also stains with the aldehyde-fuchsin method by which it is differentiated from all the other PAS positive constituents of the placenta.

6. The maternal vessels which pierce the subplacenta on their way to and from the chorio-allantoic placenta are described. The endothelium is replaced at an early stage by invasive trophoblastic elements from the subplacenta. To this modified wall of the vessels the term 'endotrophoblast' has been applied. It is vacuolated and contains PAS positive material similar to that of the subplacenta.

7. Other cellular elements of the placental site are described: the chorionic giant cells, the endodermal cells of the parietal wall of the yolk sac, and the trophoblastic and decidual giant cells of the junctional zone.

8. The evidence for the function of the subplacenta as a site of gonadotrophic (luteotrophic?) activity or as a site of transfer of materials between the decidua and the foetal tissues is discussed.

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EXPLANATION OF PLATES

Abbreviations used in the following figures:

BM basement membrane; *CS* coarse syncytium; *CY* cytotrophoblast; *D* decidua; *DC* decidua capsularis; *DG* decidual giant cell; *DS* decidual space (cavity); *E* endothelium; *EN* endotrophoblast; *FM* foetal mesenchyme; *FS* fine syncytium; *G* chorionic giant cell; *IZ* intermediate or transitional zone; *M* maternal blood sinus; *ND* necrotic zone of decidua; *P* parietal wall of yolk sac; *S* invasive syncytial outgrowth; *SG* syncytial giant cell; *SP* subplacenta; *SS* subplacental syncytium; *TG* trophoblastic giant cell; *U* uterine lumen; *UE* uterine epithelium, *V* placental vessel.

PLATE 1

(All are fixed in Bouin's fluid unless otherwise stated.)

Fig. 1. 15th day. The cytotrophoblastic layer is basophilic and extends into the decidua in the form of a letter V. The angle of the V encloses the 'central excavation' and is occupied by foetal mesenchyme. Outside the cytotrophoblastic layer is a zone of spongy syncytial trophoblast. Invasive tongues of syncytial trophoblast extend into the decidua. The decidual cavity is seen near the apex of the V of the cytotrophoblast. H. and E. ×20.

Fig. 2. Details of the floor of the 'central excavation' at 15 days. The cytotrophoblast (*CY*) is multilaminar, basophilic and shows many mitotic figures. The spongy syncytium is also basophilic but to a less degree than is the cytotrophoblast and shows no mitotic figures. Invasive tongues of syncytium are seen invading the decidua; some of these are hollow and contain maternal blood (*M*). The endoderm of the wall of the yolk sac (*P*) projects into the decidual cavity. Several chorionic giant cells in relation to the marginal syncytium are shown (*G*). H. and E. ×100.

Fig. 3. Decidua basalis at the 14th day. A multinucleate mass of syncytial protoplasm is seen invading the decidua on the outer wall of the decidual capillaries. Its cytoplasm is granular and slightly basophilic. The decidua is vacuolated and eroded. H. and E. ×440.

Fig. 4. Decidual vessel at the 15th day. The endothelium is still in evidence at *E*. The rest of the wall of the vessel has been replaced by syncytial trophoblast. H. and E. ×750.

PLATE 2

Fig. 5. 16th day. The cytotrophoblastic layer is more folded and is thicker near the floor of the central excavation (sub-placenta). The spongy syncytium is also thicker and shows many invasive tongues extending into the decidua, especially in the subplacental zone. Remnants of the decidual cavity and of the uterine lumen may be recognized deep to the subplacenta. H. and E. ×20.

Fig. 6. Details of the subplacenta from the same specimen as Fig. 5. An invasive tongue of syncytium (*S*) traverses the figure from top to bottom; it is hollow and contains maternal blood. The surrounding syncytium contains many thin-walled lacunae in which are acidophilic droplets. A chorionic giant cell is shown differentiating from the marginal syncytium. Its nucleus is large and contains clumped chromatin; the peripheral cytoplasm is acidophilic, the perinuclear cytoplasm basophilic. A few cells of the parietal endoderm are shown at *P*. H. and E. ×300.

Fig. 7. Details of the subplacenta at the 16th day. The mesenchyme of the central excavation lies at the top and left of the figure. The cytotrophoblastic layer (*CY*) is plicated and enfolds numerous maternal blood spaces. The coarse-meshed syncytium of the chorio-allantoic placenta is seen at the right of the figure (*CS*), the vacuolated syncytium of the subplacenta to the left of the figure (*SS*). Between the two types of syncytium is a transitional zone (*IZ*) with smaller lacunae containing PAS positive droplets and numerous acidophilic but PAS negative droplets. The chorionic giant cells (*G*) contain many large masses of PAS positive material. PAS and H. (after saliva). ×100.

Plate 3

Fig. 8. Transition between the coarse-meshed syncytium of the chorio-allantoic placenta (to the right) and the subplacenta (to the left). Between the two types of syncytium is a more or less solid zone of syncytium (*X*) which contains no maternal blood spaces. Small lacunar spaces with PAS positive and negative droplets appear within this zone as it is traced towards the subplacenta. A few chorionic giant cells are seen at the lower margin of the figure. PAS and H. (after saliva). ×210.

Fig. 9. Decidua basalis at the 16th day showing the large number of trophoblastic extensions of the subplacenta. The decidua between these invasive tongues is necrotic. In some areas the trophoblastic extensions appear as solid protoplasmic masses containing many nuclei. In other areas the trophoblastic extensions contain channels filled with maternal blood, probably the result of the opening up of the decidual capillaries (see Pl. 1, fig. 4). H. and E. ×200.

Fig. 10. Details of a small maternal vessel in the decidua at the 16th day. The endothelium is absent. The endotrophoblastic wall of the vessel appears cellular in some areas while in others it seems to be syncytial. There is an accumulation of PAS positive droplets in the syncytial part of the wall. A distinct basement membrane surrounds the vessel. Small extensions of the endotrophoblastic wall appear near the lower right margin of the figure and are likewise surrounded by a PAS positive basement membrane. PAS (after saliva). ×400.

Plate 4

Fig. 11. 17th day. The lateral masses of the chorio-allantoic placenta have increased in bulk and are beginning to encroach upon the central excavation. The latter is filled with foetal mesenchyme which shows angioblastic activity. The subplacenta is distinguished from the chorio-allantoic placenta by the thickness of the cytotrophoblastic layer and the large maternal blood sinuses in relation to it. The decidual cavity is conspicuous and the trophoblastic invasion of the decidua has almost reached the myometrium. The terminal ramifications of the syncytial trophoblast form a basophilic zone of degenerating giant cells (*SG*). H. and E. ×20.

Fig. 12. Margin of the subplacenta at the 18th day of gestation. Next to the central excavation (*FM*) is the richly cellular and basophilic cytotrophoblastic layer (*CY*). The typical vacuolated syncytium of the subplacenta (*SS*) merges with the coarse-meshed syncytium of the chorio-allantoic placenta (*CS*). Chorionic giant cells (*G*) and a nest of parietal endodermal cells are shown at *P*. The fold of cytotrophoblast to the left of the figure encloses an area of vacuolated syncytium which streams out into the decidua as an invasive tongue (*S*). H. and E. ×100.

Plate 5

Fig. 13. 20th day. At the top of the figure is the fine syncytium of the chorio-allantoic placenta containing maternal blood sinuses and delicate strands of foetal mesenchyme. Between it and the subplacenta is a plane of foetal mesenchyme (*FM*) from which the subplacenta is vascularized. The cytotrophoblastic layer of the subplacenta is folded and extensions of the foetal mesenchyme pass between the folds. There are also smaller, more delicate infoldings of the cytotrophoblastic layer, each containing extensions of the foetal mesenchyme. The cytotrophoblastic lamellae enclose islands of vacuolated syncytium (*SS*) which abut directly on to the decidua (*D*). The walls of a large placental vessel (*V*) are entirely replaced by syncytial trophoblast. H. and E. ×100.

Fig. 14. High-power view of the subplacenta at the 25th day showing the multilaminar cytotrophoblast, the vacuolated syncytium containing acidophilic droplets, and a vessel passing through the subplacenta, its walls being formed of syncytial trophoblast. H. and E. ×210.

Plate 6

Fig. 15. 34th day. The cytotrophoblastic layer is now thinner (compare Pl. 5, fig. 14) and the syncytium more compact. The lacunae of the syncytium, except at the margin of the subplacenta (to the right) are also smaller. The cytotrophoblastic infolding is also more complex. In the intermediate zone (*IZ*) the nuclei of the marginal syncytium of the chorio-allantoic placenta are fused into irregular, intensely basophilic chromatic masses. H. and E. ×100.

Fig. 16. 34th day. Coarse syncytium of the chorio-allantoic placenta is seen at the top right (*CS*). The cytotrophoblast (*CY*) is PAS negative. The syncytium (*SS*) close to the cytotrophoblast shows a fine PAS positive stippling. More centrally, it consists of clusters of vacuolated cytoplasm and numerous nuclei. The syncytial clusters are separated by linear aggregates of PAS positive droplets forming a geometric pattern. PAS and H. (after saliva). ×210.

Plate 7

Fig. 17. Tangential section of a placental vessel in the decidua close to the subplacenta (44 days). The endotrophoblastic wall of the vessel is spongy, the interstices containing maternal blood. It is limited externally by a PAS positive basement membrane. Clusters of trophoblastic cells, also surrounded by a basement membrane, are also found outside the main vessel and are continuous with its endotrophoblastic wall. The decidua is necrotic and contains a 'magma' of amorphous PAS positive material and cellular debris. PAS and H. (after saliva). ×220.

Fig. 18. The junctional zone between the subplacenta (to the left) and the decidua (to the right) at the 44th day. The cytotrophoblastic lamellae of the subplacenta enclose syncytial areas (*SS*) which, though closed on the placental surface, are open on the decidual surface. The PAS positive material of the subplacental syncytium is continuous with similar material in the walls of the large placental vessel lying in the decidua. PAS (after saliva). ×100.

Fig. 19. Details of placental vessel seen in Pl. 7, fig. 18. The wall of the vessel (*V*) consists of endotrophoblast (*EN*) which has replaced the endothelium. Large accumulations of PAS positive material occupy the spaces in the endotrophoblast, except in an area immediately adjacent to the maternal blood. Further out is found typical vacuolated subplacental syncytium (*SS*) containing discrete droplets of PAS positive material (at bottom of figure). PAS (after saliva). ×210.

Plate 8

Fig. 20. Details of placental vessel (44 days, which has passed through the subplacenta and is entering or leaving the chorio-allantoic placenta (to the left). The endotrophoblastic wall (*EN*) of the vessel contains PAS positive material which disappears abruptly as the endotrophoblast merges with the coarse syncytium (*CS*) of the chorio-allantoic placenta. PAS. ×220.

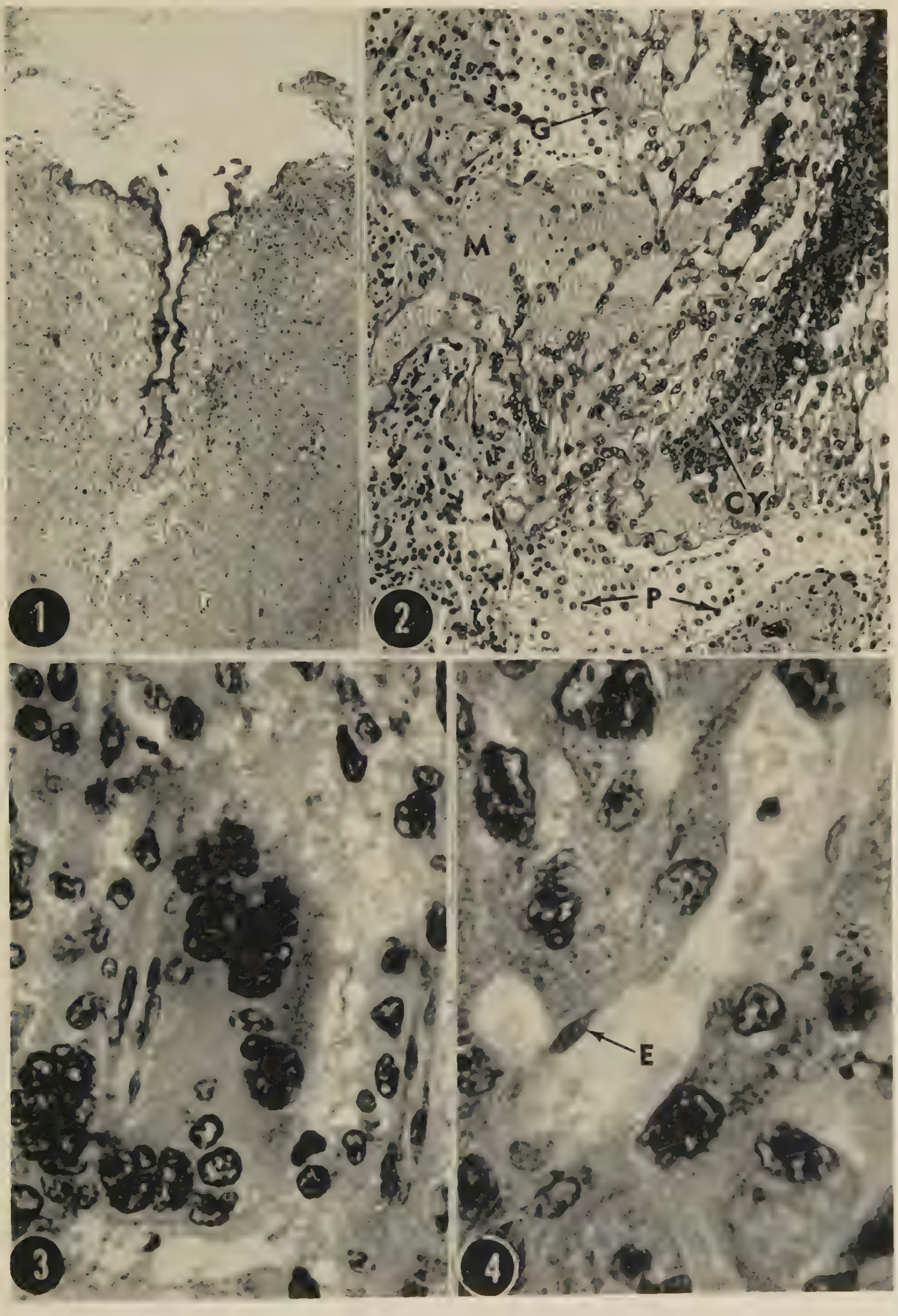
Fig. 21. Junctional zone at the 44th day. The decidua at the top left of the picture is necrotic and filled with a 'magma' of acidophilic debris (*ND*). It is permeated by invasive tongues of syncytium (*S*) in which the cytoplasm is vacuolated and perhaps degenerating. As the basal decidua is traced from its myometrial surface (bottom right) to the placental surface (top left), the cells become larger and multinucleate (*DG*) and are surrounded by individual capsules of PAS positive material. Other giant cells with basophilic cytoplasm (*TG*) are probably of trophoblastic origin. PAS and H. (after saliva). ×210.

Plate 9

Fig. 22. Section of the chorio-allantoic placenta at the 25th day showing the coarse (*CS*) and fine (*FS*) syncytium, the layer of chorionic giant cells (*G*), the layer of parietal endoderm (*P*), the decidual cavity (*DS*) and the decidua (*D*). H. and E. ×210.

Fig. 23. Parietal endoderm at the 32nd day. Note the 'frilly' character of the epithelium and the dilated intercellular spaces. Deep to the parietal endoderm is the layer of chorionic giant cells (*G*) containing clusters of PAS positive droplets. There is a well marked basement membrane between the two layers. The 'uterine milk' within the decidual cavity (*DS*) is also PAS positive. PAS and H. (after saliva). ×210.

Fig. 24. Parietal endoderm and layer of chorionic giant cells at the 32nd day. The endoderm is more compact than in Pl. 9, fig. 23, and there is less distension of the intercellular spaces. The



DAVIES, DEMPSEY AND AMOROSO—THE SUBPLACENTA OF THE GUINEA-PIG

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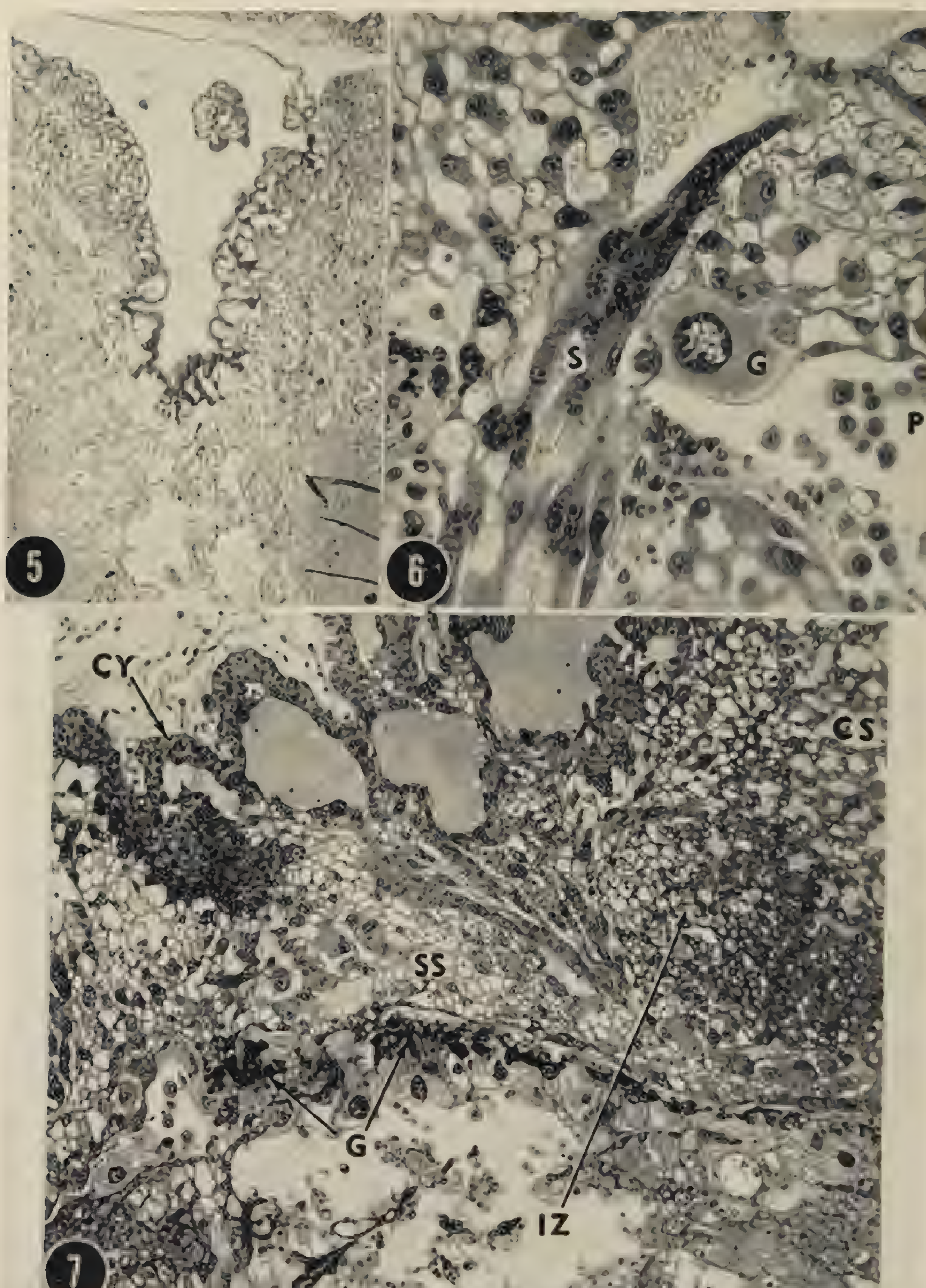

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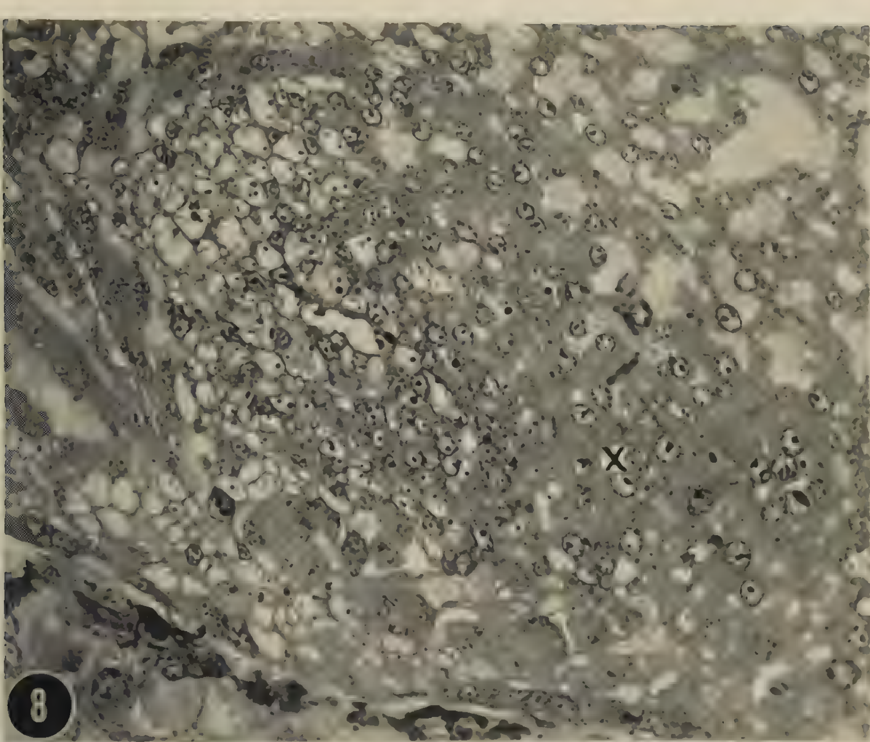

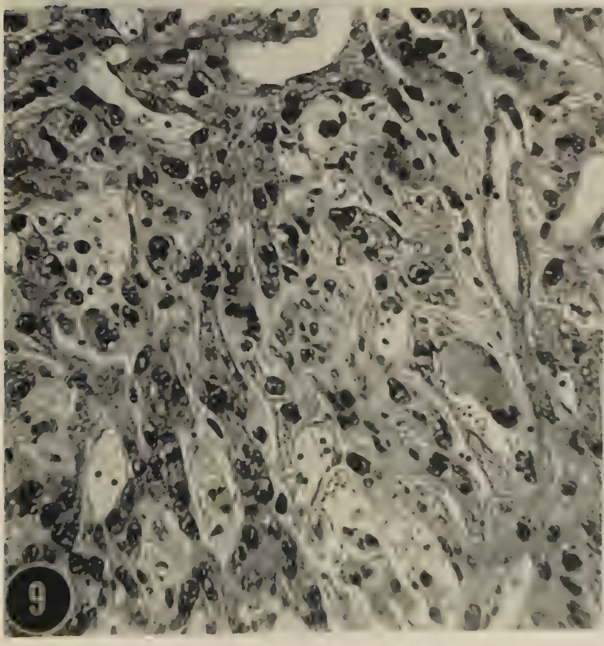

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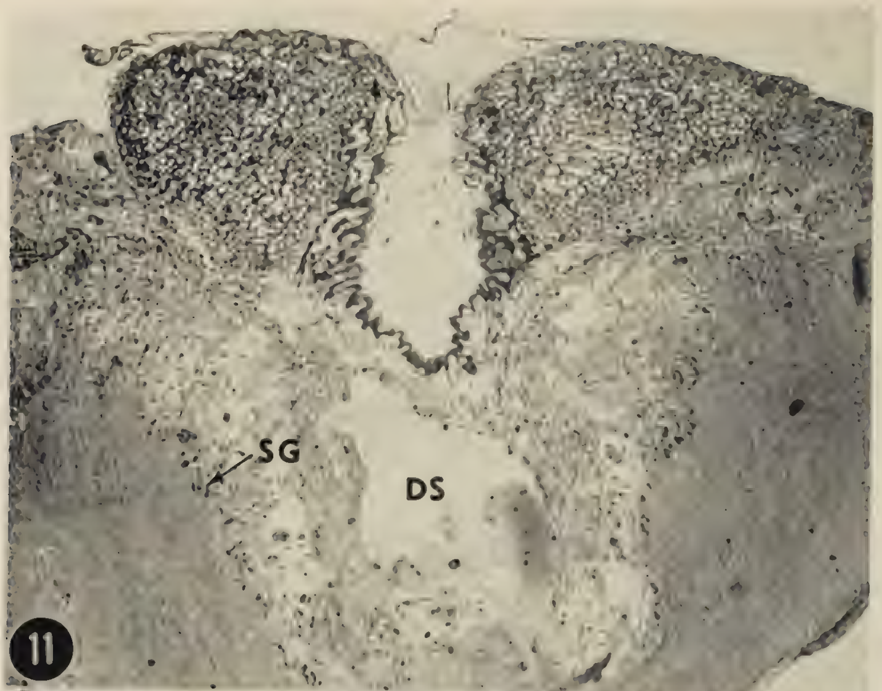

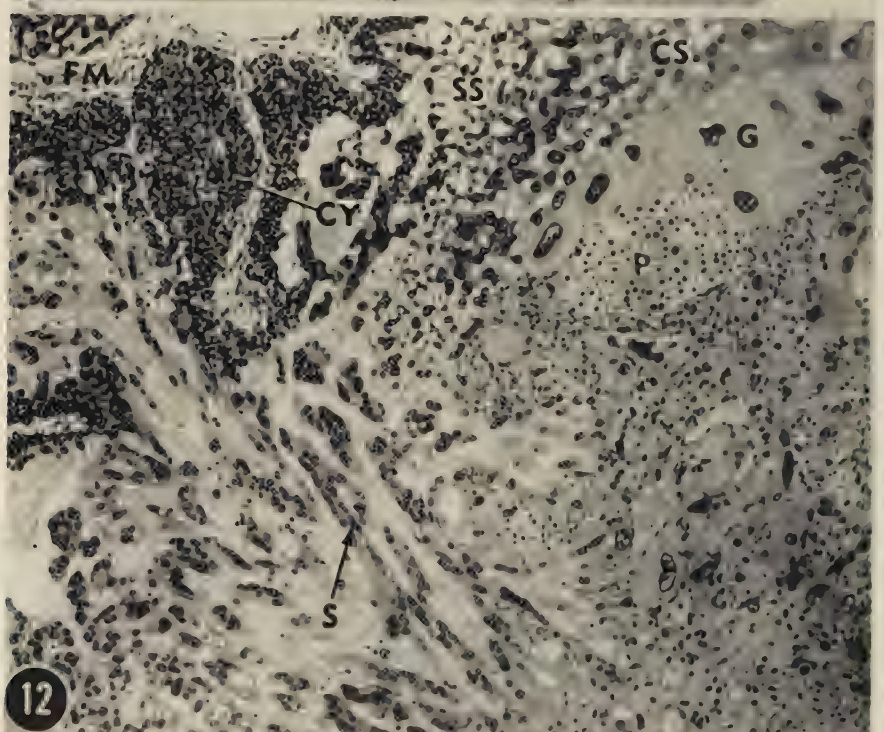

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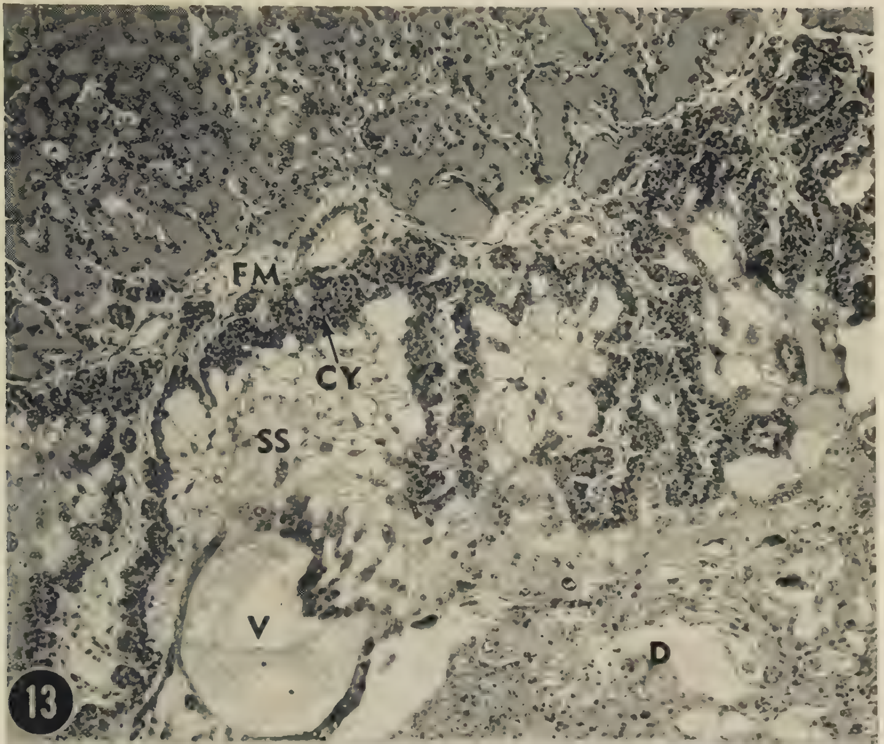

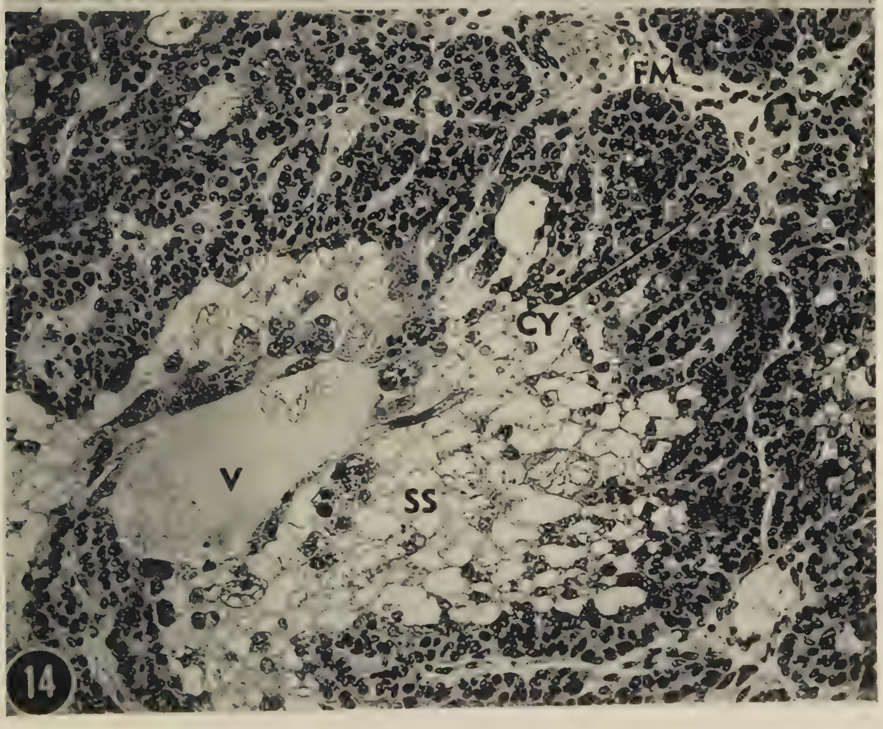

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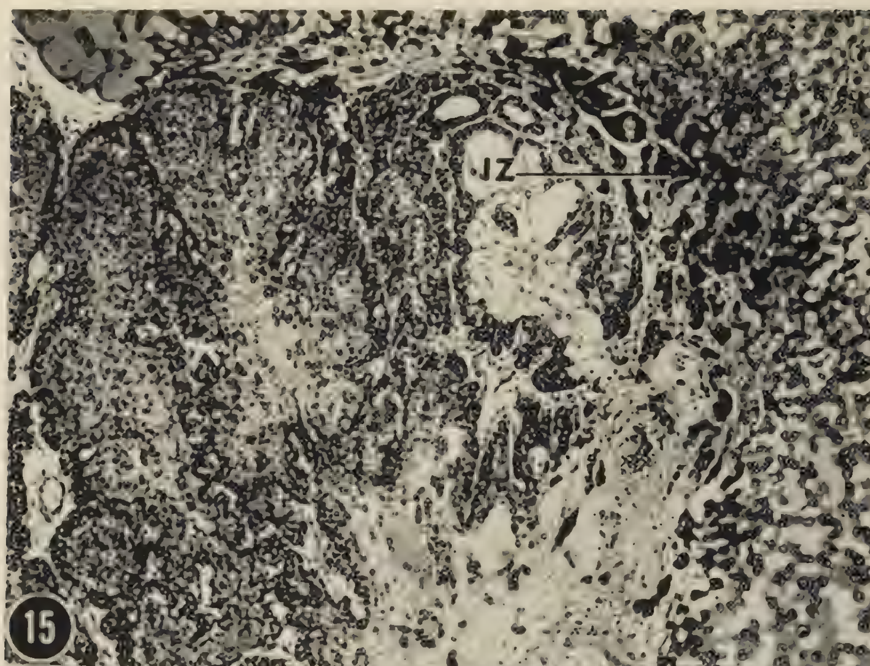

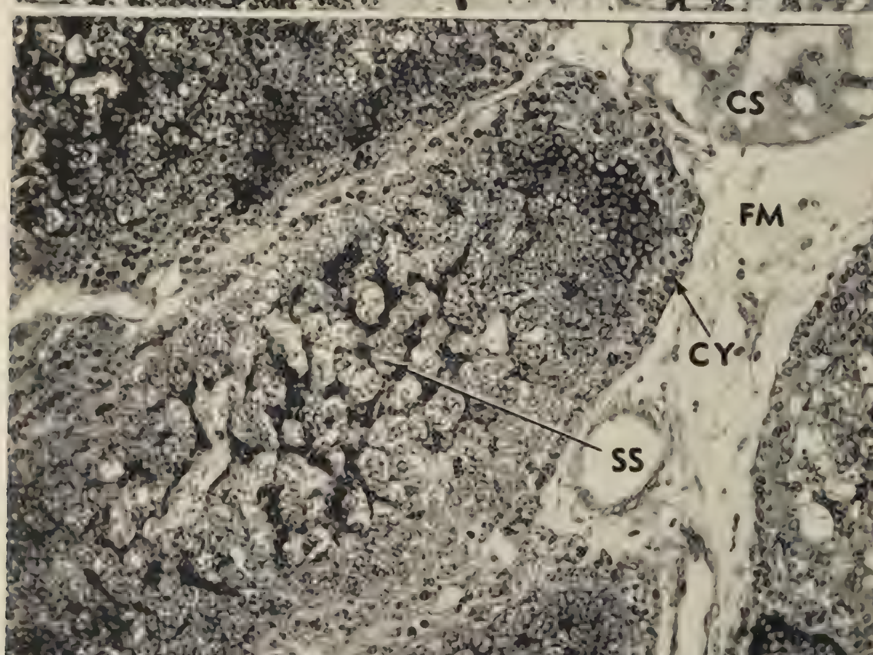

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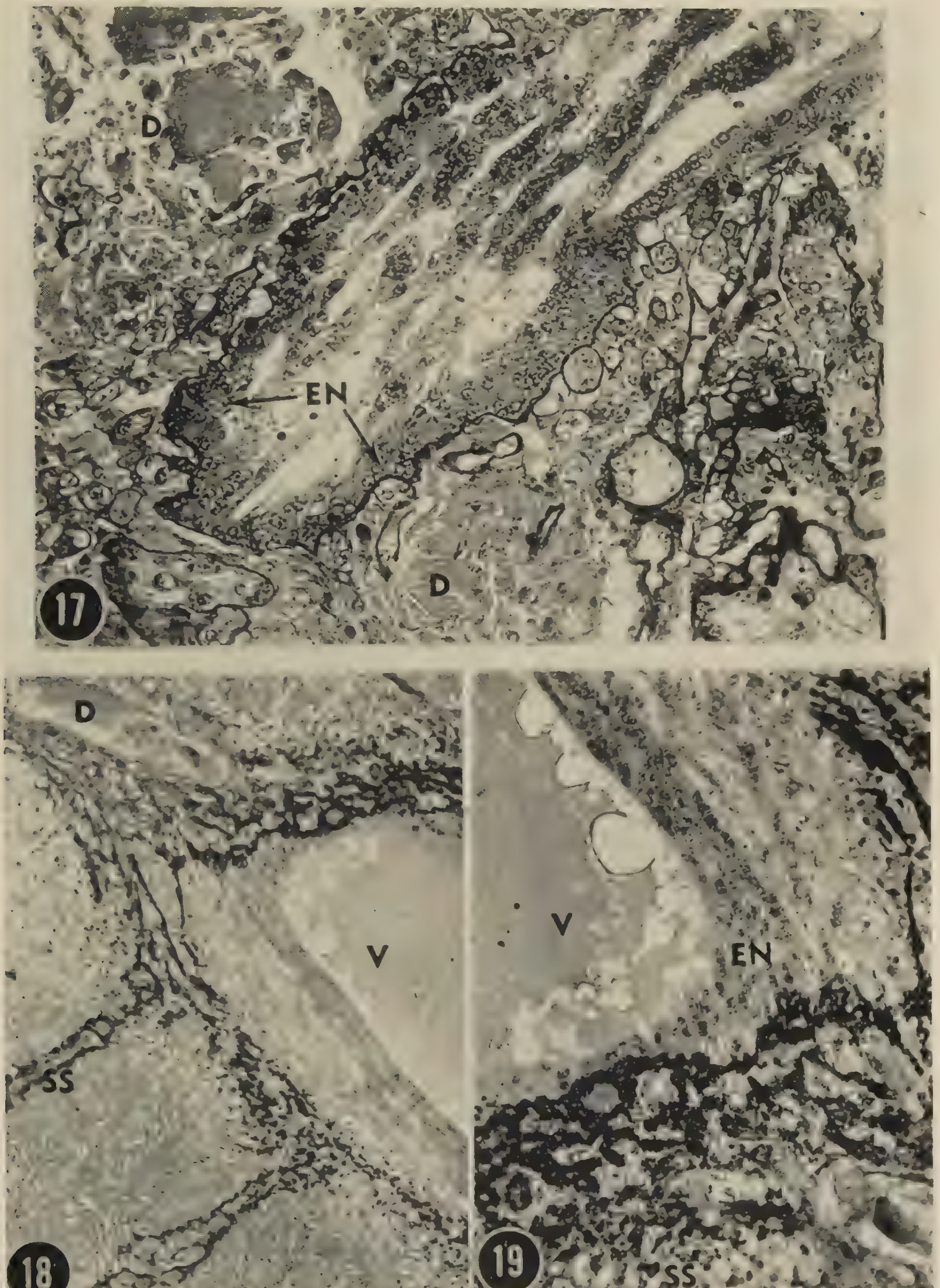

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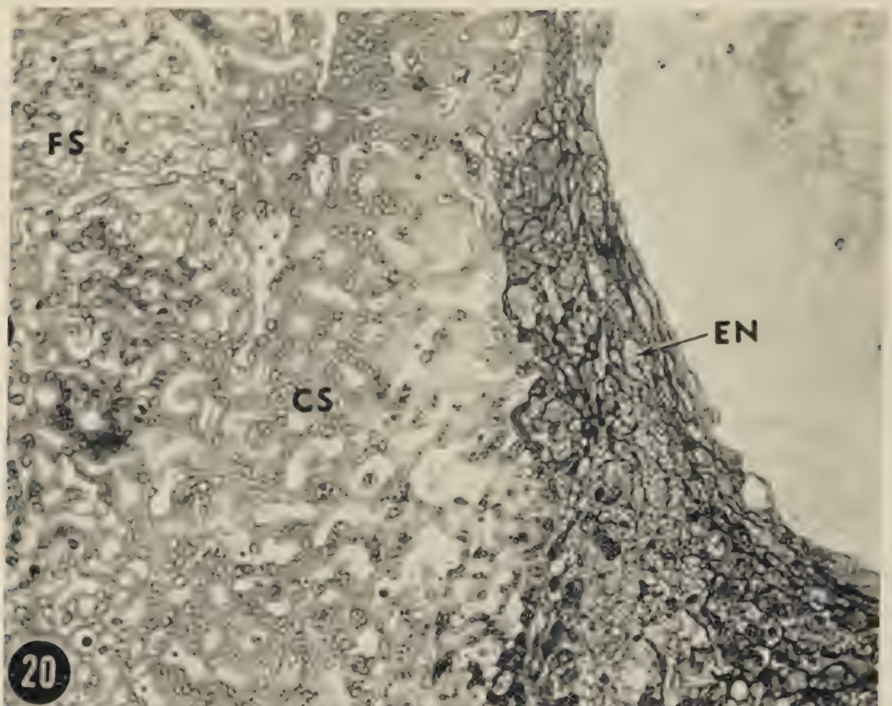

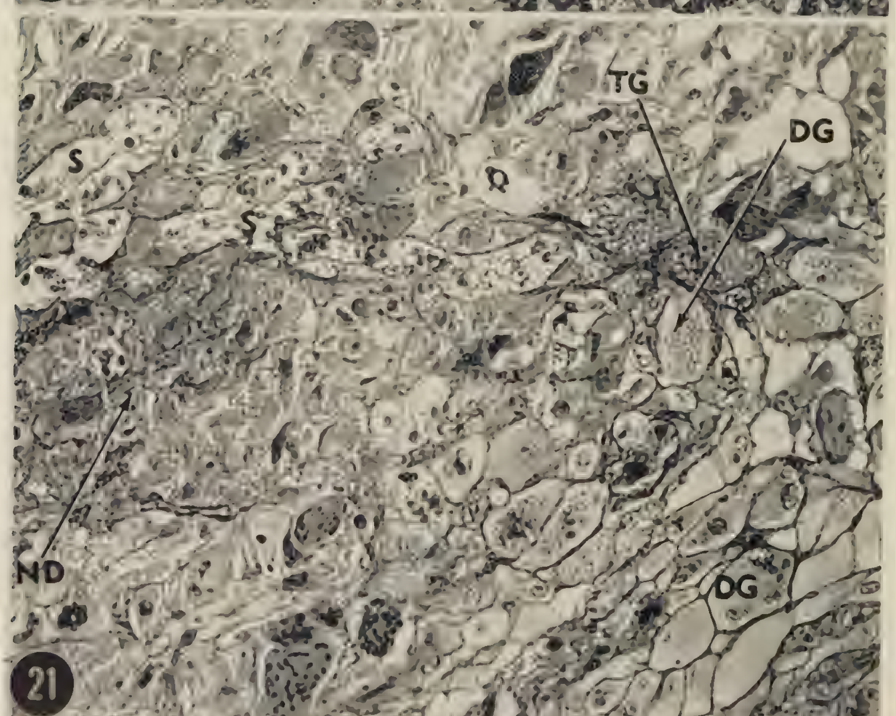

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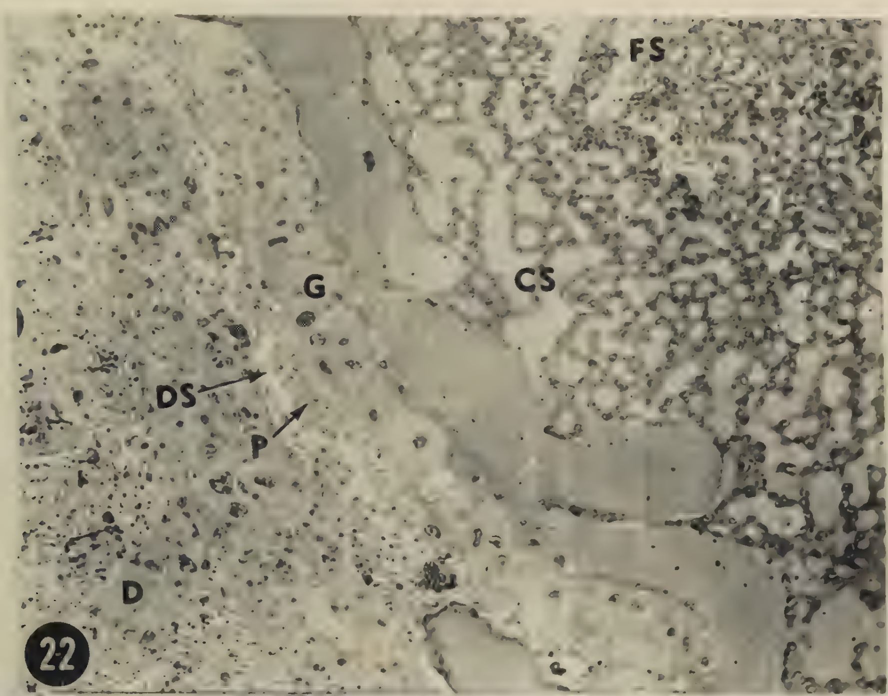

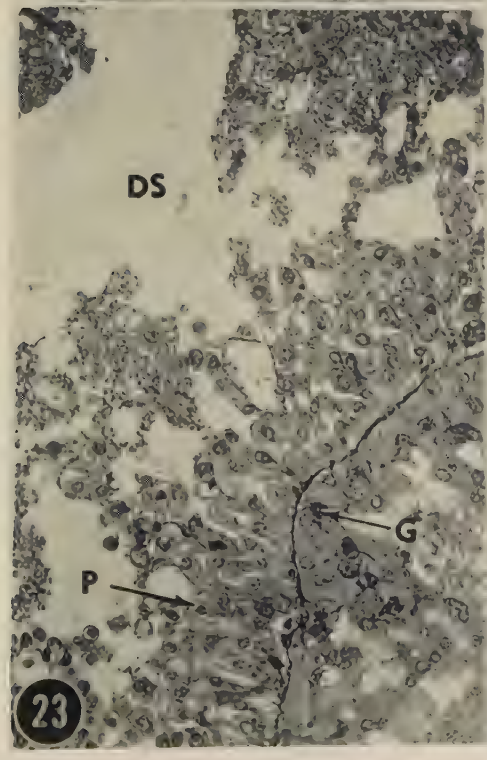

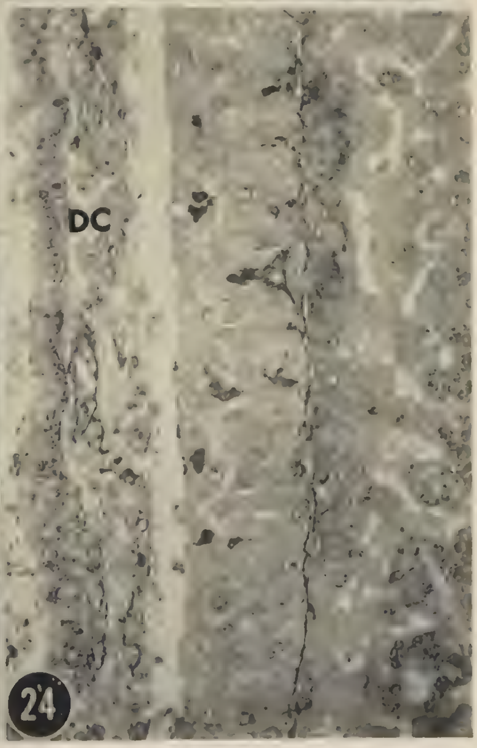

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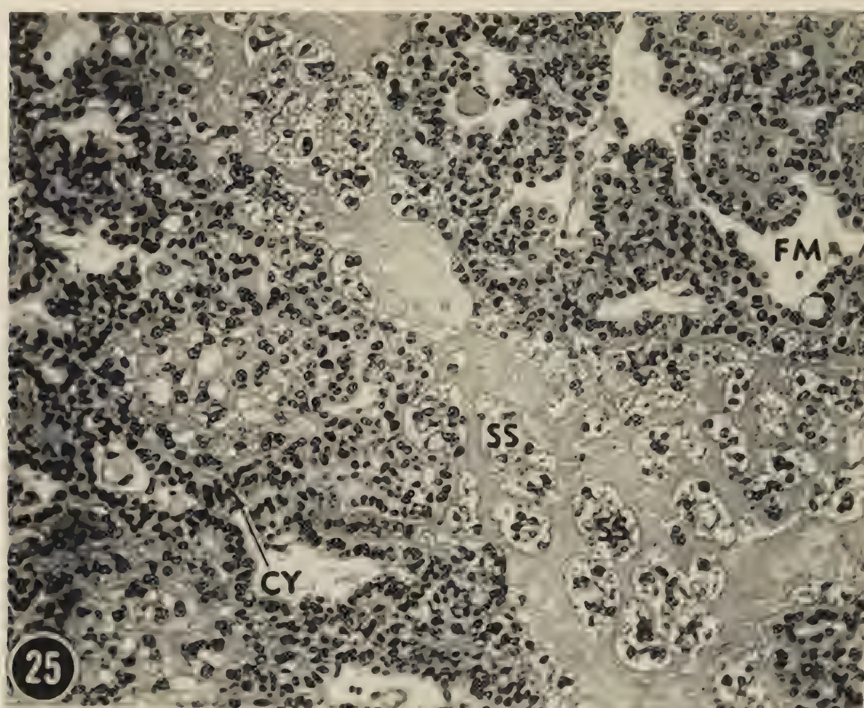

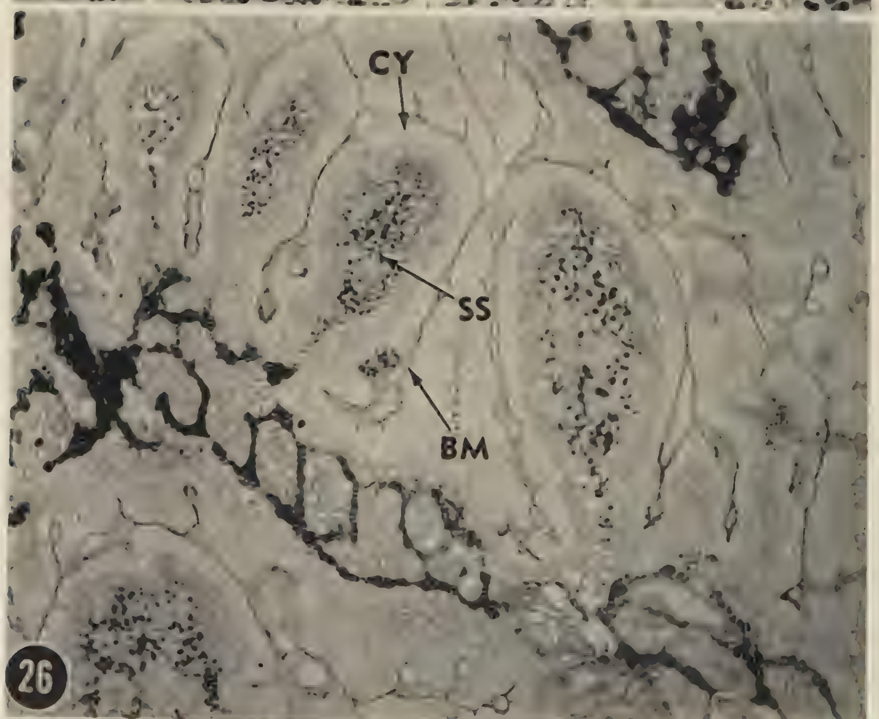

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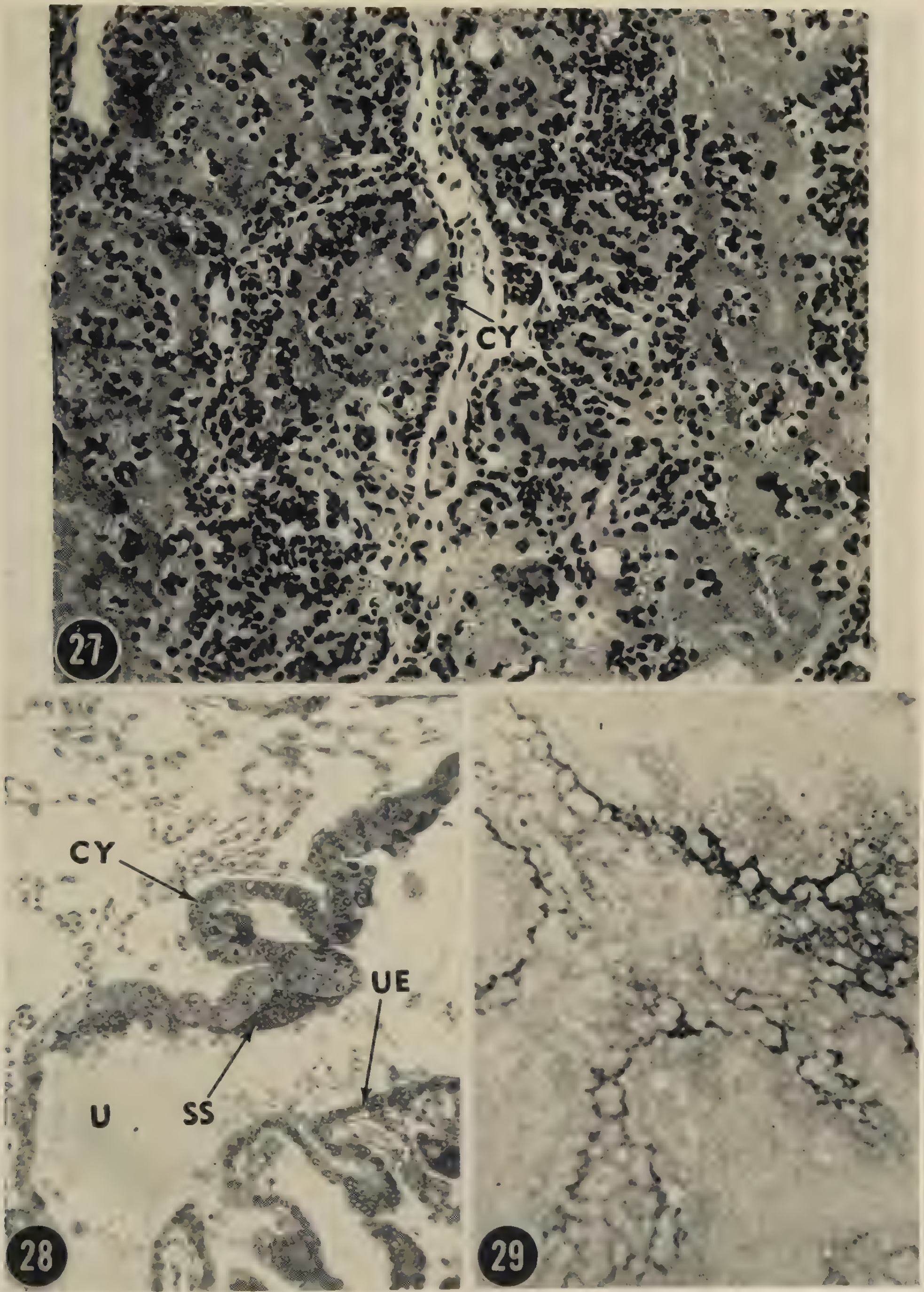

DAVIES, DEMPSEY and AMOROSO—The subplacenta of the guinea-pig

latter contain PAS positive masses continuous with the basement membrane. The chorionic giant cells contain large masses of PAS positive material. The decidua capsularis (*DC*) is seen to the left. PAS and H. (after saliva). ×210.

PLATE 10

Fig. 25. Subplacenta at the 55th day. The cytotrophoblast (*CY*) is reduced to a single layer or is absent in places, allowing the syncytium to rest directly on the basement membrane. The syncytium (*SS*) is degenerating. Between the residual syncytial islands is a mass of acidophilic (and PAS positive) material, partly amorphous and partly in droplet form. The mesenchymal septa (*FM*) are less cellular and contain few vessels. H. and E. ×210.

Fig. 26. The subplacenta at the 60th day. This preparation shows well the characteristic disposition of the PAS positive material at the height of development of the subplacenta and immediately preceding its degeneration (less advanced than in Pl. 10, fig. 25). The basement membrane stains strongly (*BM*). The cytotrophoblast (*CY*) is unstained. The syncytium (*SS*) enclosed in the cytotrophoblastic bays, contains characteristic vacuoles, each of which contains a single, shrunken droplet. More centrally in the syncytium the PAS positive droplets form a characteristic lattice-like pattern enclosing small islands of syncytium. PAS (after saliva). ×210.

PLATE 11

Fig. 27. The subplacenta at the 64th day. Degeneration is advanced. The cytotrophoblastic layer is shrunken or has disappeared over wide areas, and its nuclei are pycnotic. The syncytial trophoblast has disappeared, apart from a few degenerate clusters. The former site of the syncytium is occupied by a massive accumulation of acidophilic (and PAS positive) material. H. and E. ×210.

Fig. 28. The inter-cotyledonary chorion of a rabbit at the 13th day of gestation. This relatively simple strip of chorion unites the two lateral cotyledons of the placenta. From its position, therefore, at the floor of the 'central excavation' (top left), this area of chorion appears to be homologous with the subplacenta of the guinea-pig. The chorion here is cytotrophoblastic (*CY*) with occasional abortive masses of syncytial trophoblast (*SS*) which are deeply basophilic. The uterine lumen is seen at *U* and the uterine epithelium at *UE*. H. and E. ×210.

Fig. 29. Subplacenta of guinea-pig at 32 days. The syncytial droplets stain strongly with this stain and the characteristic pattern of droplets revealed by the method is the same as that with the PAS method (compare Pl. 10, fig. 26). Gormori's aldehyde fuchsin. ×100.

OBSERVATIONS ON THE BEHAVIOUR IN ORGAN CULTURE OF RABBIT TROPHOBLAST FROM IMPLANTING BLASTOCYSTS AND EARLY PLACENTAE

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It is generally agreed that successful implantation is the product of a balanced interaction of systemic, uterine and blastocyst factors (Proceedings of Symposia on Implantation, 1959, 1960). There is, however, a great diversity of opinion regarding the relative importance of the various factors, and the mutual relationships of blastocyst and endometrium is probably such that neither is solely responsible for the implantation phenomenon, nor is either entirely active or passive (Eckstein, Shelesnyak & Amoroso, 1959).

The study of the inter-relationships and resulting implantation when blastocysts and strips of endometrium are incubated in apposition to one another in organ culture (Glenister, 1960, 1961) should help to elucidate some problems concerning the factors controlling the growth, differentiation and invasiveness of implanting trophoblast under conditions that are more readily controllable than those obtaining in an experimental animal, and in which systemic and a number of uterine factors have been eliminated.

MATERIAL

The examination of ninety-one serially sectioned, implanted blastocysts was supplemented by a similar examination of sixty-one blastocysts that had failed to implant in identical circumstances, or were cultured by themselves without being in contact with endometrium. The details of the method have been published elsewhere (Glenister, 1961) but it should be pointed out here that the explantation of the blastocysts was carried out $6\frac{1}{2}$ days after the doe had been mated. In most instances the blastocysts were still unspaced and surrounded by a zena pellucida which was removed. The endometrial strips were removed randomly from varying points on the circumference of the uterine lumen. The cultures were incubated for periods of up to 6 days. In addition seventeen isolated portions of blastocyst were cultured by themselves for 5 days, and the embryonic discs and placentae of twenty-four implanted primitive streak and early somite embryos were explanted and cultured for periods of up to 10 days with the portion of mesometrial endometrium to which they were attached, using Shaffer's method for organ culture (Shaffer, 1956).

RESULTS

Implanted blastocysts. Their number was made up of:

(*a*) Forty-nine blastocysts that had become implanted in endometrium obtained from the doe that had conceived them or from a doe at the same stage of pregnancy.

The incubation took place in serum prepared from maternal blood (eighty-three had been explanted in this way).

(*b*) Twenty (out of forty-one) blastocysts similarly incubated but in serum prepared from the blood of an adult virgin animal.

(*c*) Nine (out of thirteen) blastocysts explanted on endometrium from an adult virgin doe but supported in serum prepared from maternal blood.

(*d*) Two (out of fourteen) blastocysts explanted on endometrium from an adult virgin doe and supported in serum prepared from the blood of an adult virgin animal.

(*e*) Eight (out of eight) blastocysts incubated on endometrial explants of maternal origin but which were placed in the culture in such a way that the deep stromal aspect of the explant was uppermost. In this way the blastocyst was in direct contact with the stroma of the endometrium without any uterine epithelium intervening.

(*f*) Three (out of twenty-two) blastocysts explanted as in (*a*) but where the pH of the medium was raised to 7·8 instead of pH 7·4 which was used for all the other experiments.

Despite the varied conditions under which these blastocysts became implanted, the attachment to and subsequent invasion of the endometrium was always effected by cellular trophoblast, irrespective of whether embryonic, abembryonic or equatorial aspects of the blastocyst surface were in contact with the endometrium. The size and shape of these trophoblastic cells was very variable, as was the size of the nuclei which were invariably vesicular with prominent nucleoli (Pl. 1, figs. 1, 5). Although superficial attachment of the blastocyst to the endometrium may occur sooner, it took from 2 to 4 days after explantation for the trophoblast to start invading the endometrium. Syncytial trophoblast was observed in many specimens, but it was usually to be found either developing over the free surface of the blastocyst (i.e. away from the implantation site) (Pl. 1, fig. 3) or extending into necrotic cellular debris lying on the surface of the endometrium. Syncytium was not observed to invade the endometrium to any depth. In three specimens a few small syncytial masses were observed in the superficial layers of the endometrium and most of them appeared to be lying in glandular crypts (Pl. 1, fig. 2). The presence of syncytium also appeared to be associated with a corresponding contact of the trophoblast with embryonic compact mesoderm or loose mesenchyme (Pl. 1, fig. 4).

In a number of the specimens the region of the endometrium to which the blastocyst had become attached was devitalized and completely acellular (Pl. 2, figs. 7, 9, 11). The depth to which these changes extended was related, in several instances, to the depth to which the trophoblast invaded. When the trophoblast came in contact with healthy living or glandular epithelium it could be seen to convert it to a symplasma (Pl. 1, fig. 5), thus giving rise to histological appearances very similar to those witnessed around normally implanting rabbit blastocysts.

All eight blastocysts incubated on the deep, stromal aspect of endometrial explants became implanted. Penetration of the endometrium by the trophoblast was just as effective in those cases as when the process started at the epithelial surface of an endometrial explant (Pl. 2, fig. 11). Thus it would seem that, under the experimental conditions described here, there was little evidence of polarity in the

endometrial components. It was also evident that trophoblast could, at least under the present experimental conditions, invade the endometrium of adult virgin does, even though this endometrium showed no sign of its histological components having been influenced by progestational hormones (Pl. 2, fig. 8).

After 4 days in culture the implanted blastocysts showed a marked tendency to collapse and to form disordered cellular plaques with little or no remains of the original cavity persisting (Pl. 2, fig. 9). Histological examination of collapsing blastocysts suggested that the collapse was preceded by a thickening of the cellular elements of the blastocyst wall, and was sometimes associated with an invasion of the embryonic disc by trophoblastic cells (Pl. 2, fig. 12). In other instances, though the cyst had collapsed the explant had gone on to give rise to well organized embryonic parts (Pl. 3, fig. 13). It is noteworthy that out of forty-nine blastocysts supported in culture by serum prepared from maternal blood only two remained as definitely expanded cysts after 5 days of culture. However, out of the twenty blastocysts cultured with serum prepared from the blood of a virgin animal ten remained vesicular after 5 days in culture, and were in fact forming expanding vesicles containing some well differentiated embryonic tissues (Pl. 2, figs. 7, 10).

When two blastocysts were explanted side-by-side on an endometrial strip, they were capable of becoming attached separately to the endometrium and, in the area where the blastocysts were contiguous, the trophoblastic cells of the respective blastocysts mingled freely (Pl. 3, fig. 14).

Control fragments of smooth muscle, explanted on endometrium from pregnant animals in exactly the same way as the blastocysts had been, sometimes became embedded in a depression on the surface of the endometrium, being connected to it by a layer of necrotic cellular debris, but no sign of invasion by muscle cells was observed. Endometrial epithelium could, however, be observed to start growing over the margins of the muscle explant.

Unimplanted blastocysts. So far, whenever a blastocyst was explanted with its zona pellucida surrounding it, the latter persisted and the blastocyst failed to implant although it appeared to be developing satisfactorily inside the membrane. Trophoblastic knobs and syncytium were often better developed in blastocysts that had failed to implant than in those that had effected the process satisfactorily. Whenever well differentiated syncytium was found in a specimen, embryonic mesenchyme or mesoderm was observed to be in contact with, or close to, the trophoblast.

The incidence of unimplanted blastocysts was raised only in those small series where they had been incubated, either in a medium with a pH raised to 7·8 (only three out of twenty-two of these blastocysts became implanted), or in contact with endometrium from a virgin doe, and also supported in a serum prepared from the blood of a virgin animal (only two out of fourteen of these blastocysts became implanted). In all the other combinations described in the previous section at least half of the blastocysts became implanted in the endometrium on which they were explanted.

Isolated portions of blastocysts. Portions obtained from the embryonic pole of blastocysts gave rise to well-differentiated embryonic structures and trophoblast, the latter showing a tendency to form syncytium as well as cellular elements in regions where there was contact with mesenchyme or mesoderm; elsewhere the

trophoblast was cellular. The few fragments obtained from the abembryonic pole of young blastocysts gave rise to trophoblast with only a few endodermal cells—no convincing mesenchyme was observed. The trophoblast in these explants was cellular and contained only a few syncytial islands. There was a marked variation in the size of the nuclei as well as of the cells of the trophoblast (Pl. 1, fig. 6), and thus they resembled closely the cells that had been observed to effect implantation in combined explants of blastocyst and endometrium (Pl. 1, fig. 1).

Primitive streak and early somite embryos and their placentae. It has been possible to keep such embryos alive and growing for 10 days, primitive streak stages going on to form neural tube, somites, gut and pulsating heart tubes (Pl. 3, figs. 15, 16). However, by the end of such a 10-day period, the embryo as a whole was no longer well organized and the aspect of the embryo that was lying on the placenta and endometrium was necrotic.

The examination of the placentae of these explanted embryos led to the observation that, irrespective of whether the embryo survived or not, the trophoblast of regions where it had maintained its contact with embryonic mesenchyme usually remained organized into cellular and syncytial components (Pl. 3, fig. 17). In specimens or regions of specimens where this contact had been lost, or had never existed, the trophoblast was cellular with little or no tendency to form syncytium (Pl. 3, fig. 18).

DISCUSSION

From the viewpoint of the experimentalist searching for information concerning the factors limiting the invasiveness, growth, histolytic effect and dissemination of trophoblast, perhaps the first reaction to the consideration of the results presented here is to echo the statement of Eckstein *et al.* (1959), that there is 'no limit to the vicissitudes to which the fertilized ovum cannot be successfully subjected'. Yet, on analysing the various results yielded by this extracorporeal approach to the problems of implantation, it will be seen that it has been possible to dissociate several of the factors that are usually intricately related and interwoven during the normal process in the intact, maternal organism.

Thus, by using opened out and flattened strips of endometrium devoid of myometrium, the 'muscular phase' of attachment described by Böving (1959*a*) has been eliminated. In addition, any possible effect on adhesion of the blastocyst to the endometrium, caused by the expansion and turgidity of the cyst with resulting increased tension within the horn, also discussed by Böving in the same paper, can be discounted as a factor in this kind of extracorporeal implantation. It is of interest to note in this context that a number of the blastocysts collapsed before subsequently becoming attached to the endometrium.

The fact that the endometrium, to which the blastocysts have become attached, has been explanted in organ culture means that any circulatory factors such as the ones described by Böving (1959*a*, *b*, *c*) can be discounted as having any influence on the implantation processes, at least in the present experimental and admittedly abnormal circumstances.

Epithelial polarity and the incidence of glands in the endometrium appear to have had no bearing on which sites have been chosen for attachment and invasion

by the trophoblast, which in this series of experiments appears to have imbedded randomly where it lay in contact with endometrium. In fact, invasion by trophoblast is very effective when attachment takes place to the under- or deep surface of an endometrial strip, where the trophoblast is not in contact with any epithelial component at all.

Induction of a decidual reaction in the endometrium (Shelesnyak, 1957, 1959, 1960) seems to play, at most, a relatively unimportant part in the experiments under consideration. The fact that implantation is effected quite satisfactorily in endometrium obtained from adult virgin animals and showing no histological evidence of decidualization, would indicate that, at any rate in the rabbit, this process may not be as essential as work in other experimental animals seems to suggest.

It has been realized (Glenister, 1961) that, as the culture media used in these experiments included serum and embryo extract, undetermined hormonal influences may have been active. The experiments of Smithberg & Runner (1956, 1960) show that only very small quantities of steroid hormones are necessary to influence implantation in pre-pubertal animals. It may be argued, therefore, that the effect at adding steroid hormones to the culture medium should have been studied in the present series of experiments. It is felt, however, that, as the media used in these experiments consisted of biological fluids, it would be more satisfactory to evolve a modification of the method involving the use of chemically defined synthetic media. This is being done and the effects of adding steroid hormones to the medium are being studied.

Experiments are also in progress to test the hypothesis propounded by Noyes (1959) suggesting that it is necessary for the trophoblast to be more mature (in terms of time since ovulation) than the endometrium presented to it for nidation.

The only uterine feature to be observed in the present experiments which can be said to have influenced the 'invasive' behaviour of the implanting trophoblast has been the vitality of the endometrium. Where the endometrium shows signs of devitalization, there does trophoblast appear to penetrate deepest, suggesting that when trophoblast-endometrium relationships are being observed during implantation active and reactive principles are being considered rather than active and passive ones; in other words, healthy endometrium would seem to offer some resistance to trophoblastic invasion, which has to be overcome by the trophoblast, possibly aided by ovarian hormonal influences.

Mossman (1937) suggests that the endometrium surrounding an implantation site becomes refractory to additional trophoblastic invasion from another blastocyst. The present work shows that blastocysts can become attached side-by-side to endometrial explants and seems to indicate that, in these circumstances, at any rate, Mossman's suggestion does not apply, thus confirming the observations of McClaren & Michie (1959).

Raising the pH of the medium from 7·4 to 7·8 though not affecting the vitality of the explants, seems to have reduced the chances of implantation. This is rather surprising considering the observations of Böving (1959*a*, *b*), which suggest that a raised pH favours adherence of the rabbit blastocyst to endometrium.

Considering now the characteristics of the cultured blastocysts and their tropho-

blast, the tendency of the implanted blastocysts to collapse after more than 3 or 4 days in culture and the apparent association in a number of instances of this collapse with destruction of the embryonic components by the trophoblast, reminds one that Maximow (1925) has noticed comparable features when observing the destruction of the embryo by its trophoblastic elements when $6\frac{1}{2}$–12-day rabbit embryos have been explanted, using the coverslip method with a lying drop, for periods of up to 6 days. An alternative interpretation is that death of the embryo precedes invasion of it by trophoblast.

The apparent difference in the behaviour of about half of the implanting blastocysts incubated in serum originating from does that have not been mated may be related, in some way, to the results of Lajos, Molnár & Görcs (1958) who have found that, in a medium containing serum and plasma from non-pregnant subjects, the connective tissue elements of human placental explants show intense growth, whereas in a medium containing serum and plasma from subjects in early pregnancy, the growth of the epithelial elements, in particular Langhan's cells, is enhanced. Again the collapsing of the blastocysts may be a manifestation of an immunological reaction, maternal serum possibly containing antibodies to the embryo (Smithies, 1959; Hirschfeld & Söderberg, 1960); or it may be explained in terms of inadequate oxygen or nutritional supply, the requirements being greater in the presence of maternal serum.

One observation in particular is noteworthy, and that is, that in all cases where the attaching trophoblast has invaded the endometrium, the trophoblast at the implantation site has been cellular in nature and has shown the phenomenon of anisocytosis. In none of the ninety-one implanted blastocysts has syncytium been observed to effect implantation and invade the endometrium to any great extent. Whenever syncytium has been observed it has usually been present either in regions in contact with serum or solid medium, or imbedded in cellular debris. Binucleated cells are the nearest approach to syncytium that has been observed to invade the endometrium to any depth. On the other hand, syncytium is often better developed in blastocysts that had failed to implant than in those that have become attached to the endometrium.

These observations culled from the results obtained under experimental conditions and supplemented by the examination of serially sectioned normal implanting rabbit blastocysts lead to the questioning of the absolute validity of a concept of 'syncytial' trophoblastic invasion in the rabbit. The examination of attaching trophoblastic knobs with high-powered light microscopes gives, at best, equivocal results, and trophoblast described by some authors as 'syncytium' can equally justifiably be said to show evidence of cell-walls between some, if not all, of the nuclei. Only when electron microscopy has been brought to bear on this problem will it be solved satisfactorily. In the mean time the argument is of more than academic interest, for it has been stated (Böving, 1959*b*) that trophoblastic invasion differs basically from cancerous invasion, in that the former is syncytial and the latter cellular.

Perhaps the solution lies in considering the diverse cellular trophoblastic elements observed to effect implantation in the intact animal, as in these experiments, as constituting a primitive kind of trophoblast, which is capable of becoming differentiated

into the cytotrophoblast and syncytiotrophoblast observed in slightly later stages. As Böving (1959*b*) has pointed out in his review of the biology of trophoblast, syncytium is a highly differentiated tissue, and the results of the present study suggest it should not be confused with the trophoblastic elements that effect attachment to, and invasion of, the endometrium during implantation.

So far, this discussion has ranged mainly over the aspects of the present series of experiments that pertain to the problems of implantation and to the characteristics of implanting blastocysts and their trophoblast. When consideration is given to the factors that influence the differentiation of trophoblast, embryonic mesenchyme and mesoderm appear, perhaps only coincidentally, to be associated with the differentiation of primitive irregular trophoblast into a well organized two-layered variety with distinct syncytial and cellular components. It may be said that this change is related to a differing nutritional environment, or to the conversion of an anaerobic environment to an aerobic one, but in the present series of experiments, whether with early blastocysts or with placentae in which the trophoblast has already differentiated into the bilaminar variety, the actual process of differentiation and the maintainance of the differentiated state seems to be associated with the presence of embryonic mesenchyme or mesoderm.

Evidence that supports the concept that mesenchyme may exert such a modulating influence on trophoblast is afforded in the first place by comparative placentology (Amoroso, 1952). In ungulate placentae (pig, sheep, cow) there is a tendency to syncytium formation at the base of the villi, i.e. nearest the foetal mesoderm, but the trophoblast covering the tips remains cellular. In the cat there is a clear association between differentiation of trophoblast to cellular and syncytial components with invasion by mesoderm, and this applies to both chorio-vitelline and chorio-allantoic portions. In the shrew, the mole and similarly in the mouse, the trophoblast of the chorio-allantoic placenta starts to produce syncytium shortly after invasion by a core of vascular allantoic mesenchyme. In the second place support can be drawn from the tissue culture and transplantation experiments of previous workers (Pljesakov, 1925; Heim, 1926, 1927, 1928; Guggisberg & Neuweiler, 1926; Neuweiler, 1927; Friedheim, 1928; Sannicandro, 1934; Sengupta, 1935; Gey, Seegar & Hellman, 1938; Gey, 1940; Jones, Gey & Gey, 1943; Knoll, 1945; Fawcett, Wislocki & Waldo, 1947; Runner, 1947; Stewart, Sano & Montgomery, 1948; Grobstein, 1949; and Thiede, 1960). There is general agreement among these workers that it is not possible to produce a pure culture of syncytium though cellular trophoblast is relatively easy to grow in cultures or transplants. The only cultures to produce syncytium are those also containing mesodermal elements. The only exception is provided by the results of Friedheim (1929) who claims to have produced pure cultures of syncytium by subculturing human Langhan's cells. This worker does not, however, indicate that he has obtained histological confirmation of his findings in living cultures; also the period of cultivation does not exceed 72 hr.

Lastly, it should be pointed out that, in 1941, Heuser & Streeter suggested that, in primates, syncytium does not differentiate till mesoderm has become related to the trophoblast, but they go on to state that the cellular trophoblast gives rise to the mesoblast as well as the syncytium. The latter part of this statement still awaits satisfactory experimental proof.

Some support for the view that trophoblast reverts to a more primitive, proliferative variety, when deprived of its normal contact with healthy foetal mesoderm or mesenchyme, is afforded by the results of Hertig & Edmonds (1940), who report that every foetal death causes deficient vascularization of placental villi, followed by trophoblastic proliferation and hydatid degeneration. Huggett & Pritchard (1945) have noted that, when rat embryos are removed or destroyed after mid-pregnancy, the trophoblast proliferates excessively before undergoing necrosis, and Fawcett (1950) has observed that, although no foetal components survive when mouse ova are transplanted beneath the kidney capsule, the trophoblast grows excessively. Lastly, it should be mentioned that McKay, Hertig, Adams & Richardson (1958) have found that anoxia causes increased proliferation although apparently not increased invasiveness of the cytotrophoblast in the human placenta.

CONCLUSION

To extrapolate a general theory, relating to the biology of trophoblast, from results obtained from the observation of rabbit trophoblast behaviour in an abnormal environment is manifestly dangerous. Yet there appears to be enough unity in the evidence gleaned from the experiments presented here, and in the evidence presented, perhaps unwittingly, by previous authors, to suggest a working hypothesis for the further investigation of the biology of trophoblast and of chorionic tumours. The concept, which is thus tentatively suggested, is that trophoblast, in its early invasive phase, consists of more primitive, mainly cellular elements exhibiting a variety of forms. These elements become differentiated into definitive cyto- and syncytio-trophoblastic layers, possibly under an organizing influence of mesenchyme, but they may revert to the more primitive, proliferative and invasive variety, when divorced from the 'restraining' mesenchymal influence.

SUMMARY

Use has been made of the organ culture technique to study the behaviour and factors controlling rabbit implanting trophoblast. The examination of ninety-one serially sectioned, implanted blastocysts has been supplemented by a similar examination of sixty-one blastocysts that have failed to implant in identical circumstances, or have been cultured by themselves without being in contact with endometrium. In addition, seventeen isolated portions of blastocyst have been cultured by themselves, and the embryonic discs and placentae of twenty-four primitive streak and early somite embryos have been explanted for periods of up to ten days with the portion of mesometrial endometrium to which they were attached. The results suggest that trophoblast, in its early invasive phase, consists of primitive, mainly cellular elements exhibiting a variety of forms. These elements become differentiated into definitive cyto- and syncytio-trophoblastic layers, possibly under an organizing influence of mesenchyme, but they may revert to the more primitive, proliferative and invasive variety when divorced from the 'restraining' mesenchymal influence.

This work has been carried out with the aid of a grant from the British Empire Cancer Campaign, which is gratefully acknowledged. I am also indebted to Dr H. B. Fell, F.R.S., and Prof. W. J. Hamilton for their help and encouragement, to Miss J. Everett for her assistance, and to Mr K. Iles for the photography.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Photomicrograph of a high power view of cellular trophoblast penetrating a materna endometrial explant and showing marked anisocytosis. The specimen was fixed 5 days after explantation. ×245.

Fig. 2. Photomicrograph of a high power view of syncytial elements emanating from an attached blastocyst 5 days after explantation on maternal endometrium. The syncytium is extending along the edge of a necrotic gland. ×268.

Fig. 3. Photomicrograph of a section through the free surface of a blastocyst fixed 5 days after explantation, and showing well differentiated trophoblastic knobs. ×215.

Fig. 4. Photomicrograph of a section through the free surface of a blastocyst fixed 6 days after explantation. A considerable amount of syncytium is being pro luced at this surface and mesenchyme can be seen to be lying deep to it. ×142.

Fig. 5. Photomicrograph of a high power view of cellular trophoblast implanting in maternal endometrium. The uterine epithelium is healthy up to the margin of the implantation site, where it becomes converted to a symplasma. Large trophoblastic cells with distinct nucleoli are seen invading the endometrial stroma deep to the epithelium and symplasma. ×284.

Fig. 6. Photomicrograph showing irregular trophoblastic cells, grown from a fragment of abembryonic blastocyst wall. The specimen was fixed 5 days after explantation. ×284.

Plate 2

Fig. 7. Photomicrograph of a section through an implanting blastocyst fixed 5 days after explantation of maternal endometrium. Columns of cellular trophoblast are seen invading acellular endometrial stroma. ×105.

Fig. 8. Photomicrograph of a section through a blastocyst which is implanting in endometrium obtained from a non-pregnant doe. This specimen contains well-differentiated embryonic tissues and structures. ×75.

Fig. 9. Photomicrograph of a section through a collapsed implanting blastocyst that had been cultured for 5 days in serum prepared from maternal blood. ×97.

Fig. 10. Photomicrograph of a section through a blastocyst obtained from the same rabbit as was the one shown in Fig. 9, but cultured for 5 days in serum prepared from the blood of a non-pregnant doe. ×30.

Fig. 11. Photomicrograph of a section through a blastocyst that had become attached to the deep or under-surface of an endometrial explant. The specimen was fixed 5 days after explantation. ×120.

Fig. 12. Photomicrograph of a section through an implanted blastocyst which had become attached to maternal endometrium by its abembryonic trophoblast. The top of the picture shows a mass of cellular trophoblast invading the remains of the embryonic disc. ×193.

Plate 3

Fig. 13. Photomicrograph of a section through a collapsed attached blastocyst which contains well-differentiated embryonic tissues 6 days after explantation. ×178.

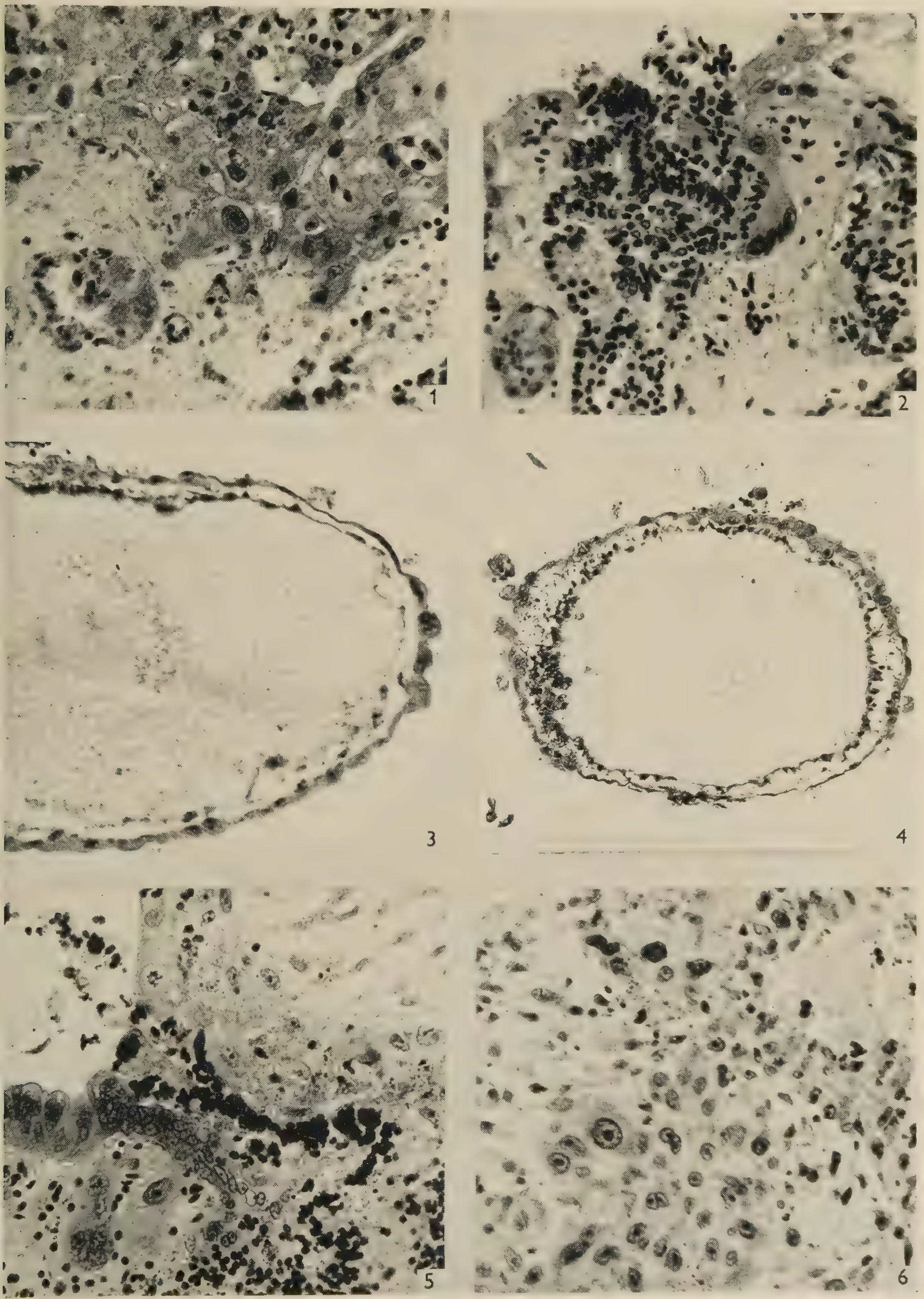
Fig. 14. Photomicrograph of a section through two blastocysts that had become attached to maternal endometrium side by side. ×70.

Fig. 15. Photomicrograph of a section through an embryo, its placenta and the endometrium to which it is attached, which was fixed 5 days after explantation. At the time of explantation the embryo was at the primitive streak stage. ×21.

Fig. 16. Photomicrograph of a section through an embryo, its placenta and the endometrium to which it is attached, which was fixed 10 days after explantation. At the time of explantation the embryo was at an early somite stage of development. The head of the embryo is seen to the left of the explant. ×19.

Fig. 17. Photomicrograph of a section through an explant of early placenta and contiguous endometrium 5 days after explantation. Mesenchymatous elements have persisted and the trophoblast is organized into a cellular and a syncytial layer. ×237.

Fig. 18. Photomicrograph of an explant comparable to the one illustrated in Fig. 17, but in which no mesenchyme has survived. The trophoblast forms a cellular plaque and no syncytium is being produced. ×148.



GLENISTER—BEHAVIOUR OF TROPHOBLAST IN ORGAN CULTURE

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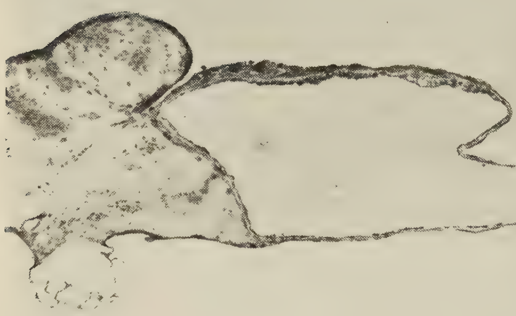

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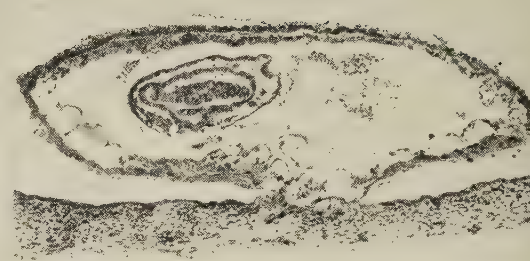

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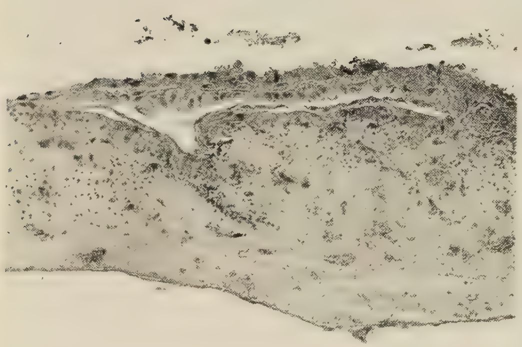

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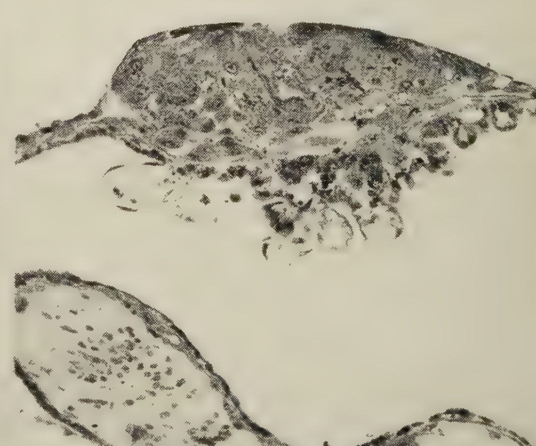

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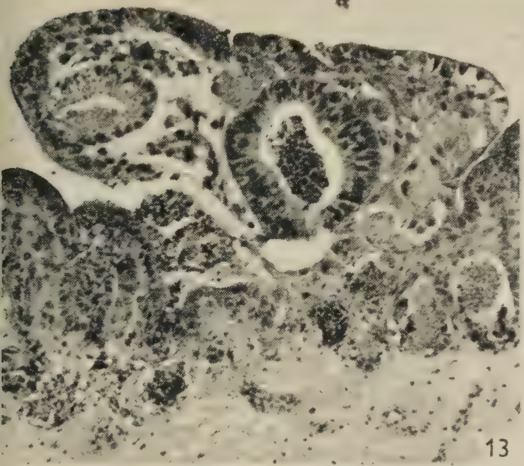
GLENISTER—Behaviour of trophoblast in organ culture



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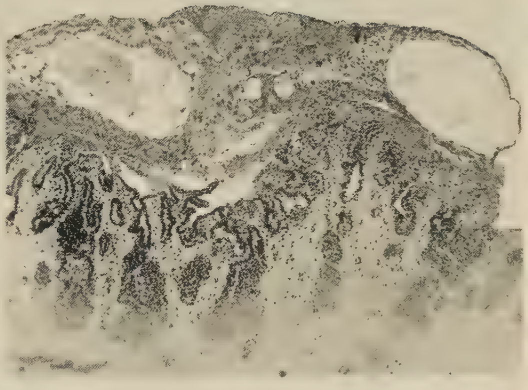

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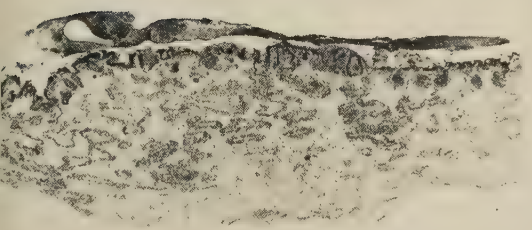

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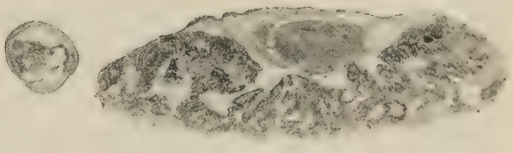

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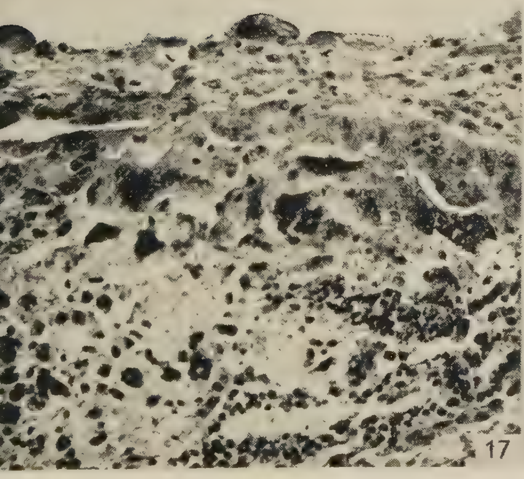

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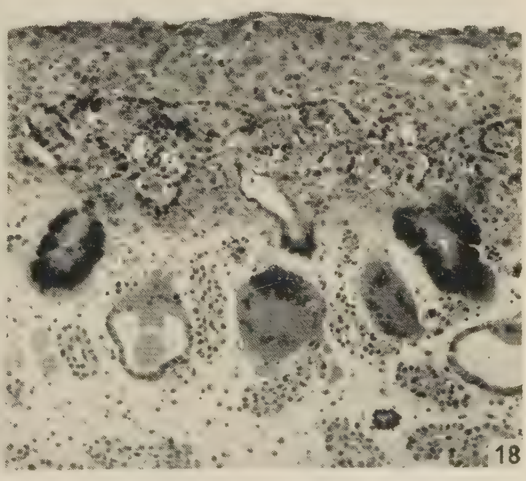

18

GLENISTER—BEHAVIOUR OF TROPHOBLAST IN ORGAN CULTURE

THE DEVELOPMENT OF THE POSTERIOR CEREBRAL ARTERY*

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There have been surprisingly few detailed accounts of the development of the arteries of the brain, and, in particular, the development of the posterior cerebral artery has never been the subject of a complete investigation, probably because this vessel develops comparatively late, at a stage when the foetus is too large to be studied by serial sections and reconstruction. The most comprehensive account is given by Padget (1948) who described and illustrated the cranial arteries in twenty-two human embryos, but even in this paper the development of the posterior cerebral artery is not dealt with adequately. The present investigation forms part of a complete survey of the embryology of the arteries of the brain and is based chiefly upon dissections of injected rat embryos, since by using this technique a relatively large number of specimens may be investigated without spending an excessive amount of time in making reconstructions.

MATERIAL AND METHODS

The course and distribution of the posterior cerebral artery in the adult black-hooded rat was investigated by the dissection of twenty animals which had been prepared by the injection of Neoprene latex 572 into the aorta followed by fixation in acidified 10 % formalin. This part of the work was necessary because I have been unable to find elsewhere an adequate description of the cerebral arteries in the rat, although a general account has been given by Greene (1935).

The technique for the injection of rat embryos has been given in detail in previous papers (Moffat, 1957, 1959) and will not therefore be described here. The injected embryos were fixed in acidified formalin and were then dissected under a binocular dissecting microscope, using watchmaker's forceps and fine glass needles. In all, 196 embryos were studied, varying in size from a C.R. length of 1·3 mm. up to full-term foetuses. In addition, five human specimens of C.R. lengths 77, 102, 131, 134 and 145 mm. were injected with Neoprene latex 572 via an umbilical artery, and were dissected after fixation in acidified formalin.

RESULTS

The posterior cerebral artery and the related vessels in the adult rat

As in man, two large vessels supply the brain on each side, namely the internal carotid and the vertebral arteries. Each internal carotid reaches the base of the brain by passing to the lateral side of the hypophysis, and after sending some small

* This work formed part of a thesis which has been approved for the award of the degree of M.D. in the University of London.

branches to the structures at the base of the brain, it gives off the posterior communicating artery, the anterior choroidal artery, a branch to the optic nerve, and the middle and anterior cerebral arteries. The posterior communicating artery runs caudally to join the posterior cerebral artery, which is a terminal branch of the basilar, thus completing the circle of Willis. Often, however, the posterior communicating artery is larger than the first part of the posterior cerebral artery, so that the latter may then be regarded as a branch of the internal carotid.

The anterior choroidal artery runs dorsally around the cerebral peduncle which it supplies, gives small branches to the region of the hippocampus and the diencephalon and ends by supplying the postero-inferior part of the choroid plexus of the lateral ventricle, anastomosing with the lateral posterior choroidal artery. The choroid plexus of the lateral ventricle consists of two parts. The main portion resembles that of man, but in the postero-inferior part of the ventricle there is an additional small rectangular tongue of tela choroidea with its own subsidiary choroid plexus which is supplied by the anterior choroidal artery. In one case, the anterior choroidal artery supplied, in addition to its usual branches, four large vessels which passed over the medial surface of the hemisphere lateral to the posterior cerebral artery, and replaced some of the branches of the latter vessel.

The terminal branches of the basilar artery are the anterior cerebellar and the posterior cerebral arteries. The anterior cerebellar artery is a large vessel which passes dorsally around the brainstem before dividing into anterior and posterior branches. The posterior cerebral artery passes dorsally into the cleft between the cerebral hemisphere and the brainstem. After a short course, it gives off from its caudal aspect, branches to the dorsal part of the mid-brain and a medial posterior choroidal artery which may be as large as the main continuation of the posterior cerebral artery. The medial posterior choroidal artery runs cranially, medial to and alongside the main posterior cerebral trunk, and gives numerous branches to the diencephalon including the pineal body. It then gives a branch which passes laterally to supply the medial side of the cerebral hemisphere where it reinforces the cranial end of the posterior cerebral artery, and finally it travels ventrally to supply the anterior part of the choroid plexuses of the third and lateral ventricles, anastomosing with the anterior cerebral artery. The next branch of the posterior cerebral artery is the lateral posterior choroidal artery which passes forwards and laterally to supply a large part of the choroid plexus of the lateral ventricle. Finally, the posterior cerebral artery continues its course on the medial side of the cerebral hemisphere, giving branches to the neighbouring regions of the cortex and eventually anastomosing with the branch of the medial posterior choroidal artery mentioned above.

Embryological findings

In embryos having a C.R. length below 4 mm. the arteries of the brain are still in a very primitive state and the brain is covered by a dense capillary plexus, except for wide midline non-vascular strips on its dorsal and ventral surfaces. Embryos, having a C.R. length between 4 and 6 mm., show several of the vessels which will later be of importance in the formation of the posterior cerebral artery, and these may be seen in Pl. 1, fig. 1, which depicts a 5·0 mm. embryo. The internal carotid artery passes to the lateral side of the developing hypophysis, and after giving off

some small branches it divides into large cranial and caudal rami. The cranial ramus passes dorsal to the optic stalk and gives off a number of branches, only one of which is of interest in the present account. This is the anterior choroidal artery, which at this stage is a small vessel running dorsally in the shallow groove between the diencephalon and the telencephalic vesicle, supplying branches to both these parts of the brain. Occasionally, the anterior choroidal artery takes origin from the proximal part of the caudal ramus. The caudal ramus passes towards the mid-brain flexure, where it gives off a large vessel which very soon divides into diencephalic and mesencephalic arteries. The former passes cranially and divides into several branches, while the latter sweeps caudally over the lateral surface of the midbrain. The caudal ramus of the internal carotid artery then passes towards the midline where it joins the corresponding vessel of the other side. The combined trunk almost immediately divides again to form the right and left anterior cerebellar arteries, which correspond to the superior cerebellar arteries of man. At this stage, therefore, the anterior cerebellar arteries are supplied by the carotid system rather than by the basilar artery, which at this stage is still unformed (Moffat, 1957).

In embryos having a C.R. length of between 6 and 9 mm. various changes in the vascular pattern have taken place, and these can be seen in Pl. 1, figs. 2 and 3. The internal carotid still divides into caudal and cranial rami and each of these still follows a course similar to that seen in the previous stage. The anterior choroidal artery still passes into the groove between the telencephalic vesicle and the diencephalon, but the former has grown caudally to cover the artery so that only the most proximal part of this vessel is visible in Pl. 1, fig. 2. In Pl. 1, fig. 3, however, the right telencephalic vesicle has been removed, together with the distal part of the anterior choroidal artery, and the whole can be viewed from the medial side. The choroid plexus of the lateral ventricle, at this early stage of its development, is represented by two shallow invaginations of the medial wall of the telencephalic vesicle—a ventral smaller and a dorsal larger invagination. These are unfortunately not visible in Pl. 1, fig. 3, but the position of the ridge which separates the two ingrowths is marked by the position of the anterior choroidal artery which runs along it, giving small branches to the adjacent surfaces of the diencephalon and the telencephalon. The cranial end of the anterior choroidal artery breaks up into branches which anastomose with the end of the developing anterior cerebral artery.

The caudal ramus of the internal carotid artery gives off the common stem of origin of the mesencephalic and diencephalic arteries, but two new vessels have now appeared in this region. One of these, the posterior choroidal artery, arises from the common stem just proximal to the diencephalic artery, although occasionally it has a separate origin from the caudal ramus. In Pl. 1, fig. 2, it can be seen to disappear behind the expanding telencephalon. Pl. 1, fig. 3, however, shows how this vessel breaks up into branches on the lateral wall of the diencephalon, anastomosing with branches of the anterior choroidal artery. Occasionally, the posterior choroidal artery may take origin separately from the caudal ramus of the internal carotid artery. Arising from the common stem or from the mesencephalic artery itself is a small, but remarkably constant, vessel which will be referred to as the accessory mesencephalic artery. The diencephalic and mesencephalic arteries remain

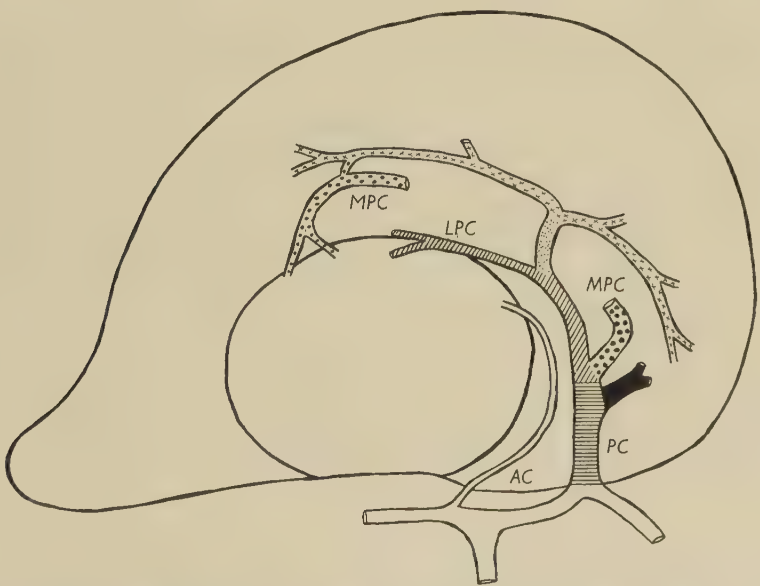
prominent, and Pl. 1, fig. 3, shows particularly well the manner in which these two vessels curve cranially and caudally respectively.

The next stage which is of interest in the present study occurs in embryos of a C.R. length between 9 and 12 mm. and is marked by the development of one or more large branches of the anterior choroidal artery which supply the medial wall of the posterior part of the telencephalic vesicle. Pl. 1, fig. 4, shows the vessels lying in relation to the medial wall of the right telencephalic vesicle and is taken from an 11·7 mm. embryo, while Pl. 2, fig. 5, represents both telencephalic vesicles of a 12·0 mm. embryo. The diencephalic and posterior choroidal arteries both still end by ramifying on the side wall of the diencephalon and are therefore not seen in these specimens. The anterior choroidal artery is prominent and it gives a large branch which passes dorsally, dips into the shallow choroidal fissure, and then breaks up on the wall of the telencephalic vesicle. This branch may become larger than the continuation of its parent vessel (Pl. 1, fig. 4). Often, two or more branches are present in this situation. The branches of the anterior choroidal artery form a rather dense plexus which is beginning to show signs of a longitudinal arrangement, and it is in this plexus that the distal part of the posterior cerebral artery will develop. As well as the main choroidal fissure there is still a much shorter, more ventral fissure which will give rise to the accessory choroid plexus in the adult. The main stem of the anterior choroidal artery still runs along the ridge between the two fissures and gives branches to each. Its terminal branches communicate with the terminal branches of the anterior cerebral artery, which supply the cranial extremity of the choroidal fissure. Numerous branches are also given off to the region of the diencephalic–telencephalic junction.

In embryos having a C.R. length of over 12 mm., the anterior choroidal artery loses its large telencephalic branch or branches, the longitudinal plexus on the medial wall of the telencephalon acquiring a new vessel of supply derived from the posterior choroidal artery and another, shortly afterwards, from the diencephalic artery. Pl. 2, fig. 6, shows the medial wall of the right telencephalic vesicle in a 14 mm. embryo, in which the posterior choroidal artery has been stripped from the side of the diencephalon and left attached to the telencephalon. The anterior choroidal artery still gives a few very small branches to the longitudinal plexus on the medial wall, but the main vessel of supply to this plexus is now a new laterally directed branch of the posterior choroidal artery which crosses the narrow space between the diencephalon and the telencephalon. The distal part of the posterior choroidal artery still gives some small diencephalic branches but ends by anastomosing with the cranial end of the anterior choroidal and with the descending terminal branch of the anterior cerebral artery and gives a number of small, laterally directed branches into the choroidal fissure. Part of the plexus on the medial side of the telencephalon now has a short segment of a single vessel running through it, and this will form the terminal portion of the posterior cerebral artery. Between this vessel and the anterior cerebral artery the plexus shows a particularly dense area, and this marks the point where a small branch of the diencephalic artery runs laterally to join the plexus. The choroidal fissure cannot be seen in the photograph since at this stage it is no longer a wide groove into which dip the large vessels of the telencephalon, but is now reduced to a narrow slit through which only very small vessels can pass.

These vessels are derived from the anterior cerebral, the posterior choroidal and the anterior choroidal arteries. The terminal branches of the anterior choroidal artery now supply only the posterior part of the choroidal fissure and they no longer anastomose directly with the anterior cerebral artery.

A slightly later stage is seen in Pl. 2, fig. 7, which shows the medial side of the right telencephalic vesicle in a 21·8 mm. embryo. The telencephalic branch of the posterior choroidal artery is now large and forms part of the posterior cerebral artery, which continues on the medial wall of the telencephalon. Eventually, this telencephalic branch will become larger than the continuation of the posterior choroidal artery so that the latter vessel, in adult terminology, will become a branch of the posterior cerebral artery, and will, in fact, form the lateral posterior choroidal artery. The diencephalic artery is large, and after giving branches to the



Text-fig. 1. Diagram of the medial surface of the right cerebral hemisphere to show the components of the posterior cerebral artery. A segment of the diencephalic artery has been removed. Horizontal hatching: common trunk of origin of brainstem arteries; oblique hatching: posterior choroidal artery; heavy stipple: diencephalic artery; light stipple: lateral branch of posterior choroidal artery; small crosses: new vessel from plexus on medial wall of telencephalic vesicle; solid black: mesencephalic artery. *AC*, anterior choroidal artery; *PC*, main stem of posterior cerebral artery; *MPC*, adult medial posterior choroidal artery; *LPC*, adult lateral posterior choroidal artery.

diencephalon, it gives off its telencephalic branch and then passes ventrally to anastomose with one of the terminal branches of the anterior cerebral artery. In Pl. 2, fig. 7, the diencephalic artery has been divided so that only its telencephalic branch (which reinforces the cranial end of the posterior cerebral artery) and its terminal descending portion are visible and the latter, in this photograph, appears to be a continuation of the posterior cerebral. The diencephalic artery persists into adult life as the medial posterior choroidal artery which supplies the choroid plexus

of the third and lateral ventricles, and sends a laterally directed branch to reinforce the posterior cerebral artery. At this stage, therefore, the choroid plexus is supplied by the anterior cerebral, the diencephalic, the posterior choroidal and the anterior choroidal arteries, from before backwards, and this arrangement persists into adult life.

To sum up, the definitive posterior cerebral artery (Text-fig. 1) is formed proximally from part of the caudal ramus of the internal carotid artery followed by the elongated common trunk of the mesencephalic, diencephalic and posterior choroidal arteries. The intermediate portion is formed by the proximal portion of the original posterior choroidal artery, the distal portion of this vessel becoming the adult lateral posterior choroidal artery. The distal portion is formed by the lateral branch of the original posterior choroidal artery, together with a vessel which develops from the plexus on the medial wall of the telencephalic vesicle. The diencephalic artery forms the medial posterior choroidal branch of the posterior cerebral artery while the mesencephalic and accessory mesencephalic arteries form its midbrain branches.

The five human specimens showed many resemblances to the rat embryos, and in three of these, namely those of 77, 102 and 134 mm., the arrangement of vessels was very similar to that shown in Pl. 2, fig. 7. The vessel which was recognizable as the posterior cerebral artery gave off mesencephalic branches, a large diencephalic branch, a choroidal branch (the lateral posterior choroidal artery) and finally ended by supplying the medial aspect of the posterior pole of the brain. The diencephalic branch supplied the brain stem and ended by bridging the gap between diencephalon and telencephalon and supplying the medial wall of the latter. It also supplied a choroidal branch in the 72 and 102 mm. embryos but this could not be traced in the 134 mm. specimen, possibly because the injection was incomplete. At 131 and 145 mm., the diencephalic artery no longer supplied the telencephalon but ended as a choroidal branch. The loss of the telencephalic branch appears to be due to the enormous growth in a caudal direction of this part of the brain.

DISCUSSION

The adult human posterior cerebral artery bears a fairly close resemblance to the corresponding artery in the rat, although there are a few points of difference. In the human, in the 'normal' circle of Willis, the posterior cerebral artery arises from the basilar artery and is twice the size of the posterior communicating artery (Padget, 1945), whereas in the rat the vessel usually receives its blood from the carotid and basilar arteries in approximately equal proportions. In the rat, the medial posterior choroidal artery (i.e. the vessel derived from the diencephalic artery) supplies a branch to the medial wall of the cerebral hemisphere which anastomoses with the cranial extremity of the posterior cerebral artery. A rather similar state of affairs occurs in the rabbit, in which the posterior cerebral artery gives off a large branch which supplies mesencephalic and diencephalic derivatives and which ends by giving a few branches to the medial side of the cerebral hemisphere (Nilges, 1944).

The anterior choroidal artery in both the rat and the human embryo appears at an early stage and in both species it gives branches to the diencephalon before passing to the choroidal fissure. It is described, or illustrated, by His (1904), Mall

(1904), de Vriese (1905), Evans (1912), Thyng (1914) and Padget (1948, 1956). There are, however, no previous references to its importance in providing the main blood supply to the caudal pole of the telencephalic vesicle in the early stages of development, although His notes that the choroidal fissure is at first very wide and that the vessels of the choroid plexus are at first only a part of the general network on the medial wall of the telencephalic vesicle.

Previous accounts of the development of the posterior cerebral artery have been vague and inconclusive. Mall states that it is formed from 'all of the branches together arising from the circle of Willis between the third and fourth nerves behind and the origin of the middle cerebral in front...'. Thyng, in his plate 2, shows a loop passing from the caudal ramus of the internal carotid from which arise four branches to the midbrain and diencephalon, and this is labelled the posterior cerebral artery. Bremer (1943) discusses Thyng's embryo and suggests that these multiple branches become the posterior cerebral artery by the absorption of all but one of the capillary roots by which they arise. Padget (1948) notes that the posterior cerebral artery could not be definitely identified in her series of human embryos (from 3 mm. up to 43 mm.), but states that its distal part emerges 'by means of an elaboration of one of the large diencephalic or mesencephalic branches of the posterior communicating artery...'. In a later paper, however (Padget, 1956), she states that 'the prominent (dorsal) diencephalic artery of the embryo, from which the posterior cerebral artery arises, is represented by at least one adult posterior choroid artery'.

The findings in the five human specimens described above suggest that the development of the posterior cerebral artery is similar in both rat and man, and if this be accepted, many of the anomalies of the vessel in the human adult can be explained. The persistence of the embryonic condition in which the posterior cerebral artery is fed mainly by the caudal ramus of the internal carotid artery is of course well known. The posterior cerebral may occasionally be replaced by a branch of the anterior choroidal artery (Adachi, 1928; von Mitterwallner, 1955). Since the anterior choroidal forms the main blood supply to the occipital pole of the brain at one stage of development, it is easy to see how it may form the stem of the posterior cerebral artery if the telencephalic branch of the embryonic posterior choroidal artery does not take over the supply of the hemisphere. A double posterior cerebral artery is met with infrequently (Windle, 1888; Longo, 1905; Gordon-Shaw, 1910; von Mitterwallner, 1955; Alpers, Berry & Paddison, 1959). This is surprising, since the condition is presumably due to one of the brainstem vessels arising independently instead of from the common trunk and this is often seen in rat embryos, and in the human embryos illustrated in Padget's paper. The close association between the anterior and posterior choroidal arteries in the embryo explains the important anastomoses which link these two vessels in the adult which have been described by numerous authors.

The development of the posterior cerebral artery in the rat is an interesting example of the Law of Recapitulation. The present investigation has shown that the blood supply of the caudal part of the telencephalon is derived first from a branch of the cranial ramus of the internal carotid, then from the posterior choroidal artery. The latter is later reinforced by the diencephalic artery and finally, a branch of the

basilar artery may form the stem of origin of the posterior cerebral artery. In this way, it can be seen that as the telencephalon grows caudally, it receives its blood supply from vessels which take their origin from progressively more caudal sources. Abbie (1934) has shown that a similar caudal migration of the origin of the posterior cerebral artery has occurred during the phylogenetic history of this vessel in response to the backward growth of the telencephalon. In reptiles, the posterior cerebral artery takes its origin from the cranial ramus of the internal carotid, and as the evolutionary scale is ascended, the artery utilizes other, more posterior parts of the primitive network to form its stem of origin until, in the primates, it has consolidated its most posterior stem of origin in the 'anterior midbrain channels'. A similar caudal displacement of the origin of the posterior cerebral artery was noted by Hoffmann (1900), who, in fact, gave four possible positions for the origin of the vessel in different species.

SUMMARY

1. The development of the posterior cerebral artery has been studied in 196 injected rat embryos.

2. The arterial supply to the medial aspect of the posterior part of the telencephalon is at first provided by one or more large branches of the anterior choroidal artery.

3. At a later stage the posterior choroidal artery gives off a large lateral branch which bridges the gap between the diencephalon and telencephalic vesicle and takes over the supply of the plexus on the medial wall of the vesicle from the anterior choroidal artery.

4. The distal portion of the posterior cerebral artery develops as a longitudinal vessel derived from this plexus and it is reinforced at its cranial end by a laterally directed branch of the diencephalic artery.

5. The adult posterior cerebral artery is thus derived from a part of the posterior communicating artery, the common stem of origin of the posterior choroidal, diencephalic and mesencephalic arteries, the proximal part of the posterior choroidal artery, its lateral branch, and a new vessel which develops in the plexus on the medial wall of the telencephalic vesicle.

6. The distal part of the embryonic posterior choroidal artery persists as the adult lateral posterior choroidal artery, while the diencephalic artery forms the medial posterior choroidal artery.

I should like to express my thanks to Mr A. Welch who is responsible for the photography, and to Miss Valerie Mahoney for technical assistance and for drawing the diagram.

I should also like to acknowledge here the help, advice and encouragement which I received during the course of this work from the late Prof. J. S. Baxter.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Right side of the cranial region of a 5 mm. embryo ($\times 24$). The cranial ramus of the internal carotid passes dorsal to the optic stalk, which has been divided. The caudal ramus passes ventral to the midbrain flexure where it gives off by a common trunk the diencephalic and mesencephalic arteries.

Fig. 2. Left side of the cranial region of a 6·9 mm. embryo ($\times 17$). The plexiform middle cerebral artery is developing on the side of the telencephalic vesicle, which hides all but the most proximal portion of the anterior choroidal artery. At the midbrain flexure, the caudal ramus of the internal carotid artery gives off a common trunk which divides into posterior choroidal, diencephalic and mesencephalic arteries.

Fig. 3. Right side of the mid- and fore-brain of a 7·0 mm. embryo ($\times 17$). The right telencephalic vesicle has been detached and turned over to display its medial surface with the anterior choroidal (arrowed) and anterior cerebral arteries.

Fig. 4. Medial surface of the right telencephalic vesicle of an 11·7 mm. embryo ($\times 23$). The anterior choroidal artery arises from the cranial ramus of the internal carotid artery and gives off a large branch which supplies a longitudinal plexus on the medial wall of the hemisphere.

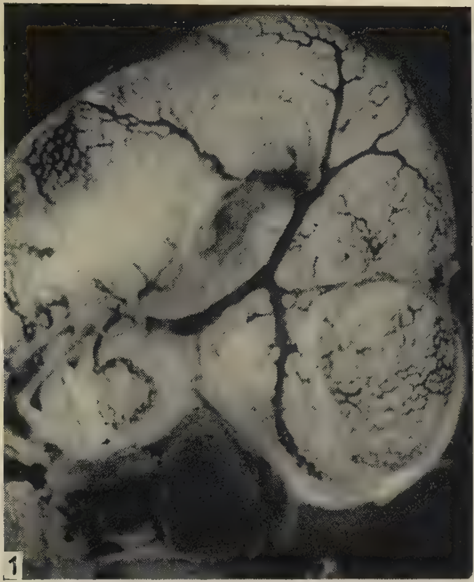
PLATE 2

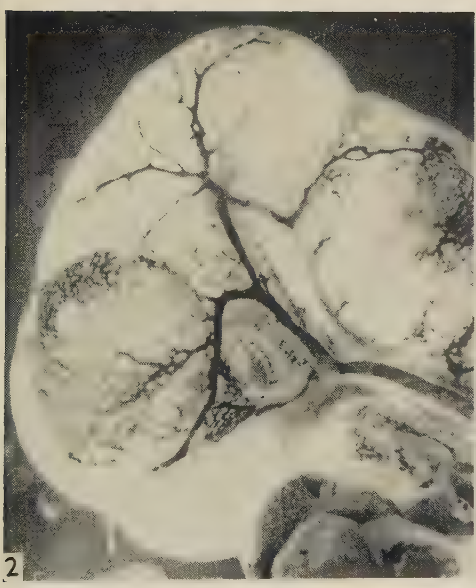
Fig. 5. The medial surfaces of the right and left cerebral hemispheres of a 12·0 mm. embryo ($\times 32$). The caudal poles are towards the centre of the photograph. The anterior cerebral artery is a single midline vessel for most of its course, and has remained adherent to the left hemisphere. On both sides it gives a descending branch to the cranial end of the choroidal fissure. The

anterior choroidal artery also supplies the fissure, but its largest branch on each side supplies the plexus on the medial wall of the hemisphere.

Fig. 6. Medial surface of the right hemisphere of a 14·0 mm. embryo ($\times 23$). The posterior choroidal artery has been removed from the side of the diencephalon and remains adherent to the specimen. Its lateral branch (arrowed) has now taken over the supply of the plexus on the medial wall of the hemisphere in which part of the posterior cerebral artery can be seen.

Fig. 7. Medial surface of the right cerebral hemisphere of a 21·8 mm. embryo ($\times 18$). The choroid plexus is supplied, from before backwards, by the anterior cerebral, diencephalic, posterior choroidal and anterior choroidal arteries. The lateral branch of the posterior choroidal artery (slightly out of focus) now forms part of the posterior cerebral artery which continues on the medial wall of the hemisphere and which is reinforced at its cranial end by a branch of the diencephalic artery.





MOFFAT—THE DEVELOPMENT OF THE POSTERIOR CEREBRAL ARTERY

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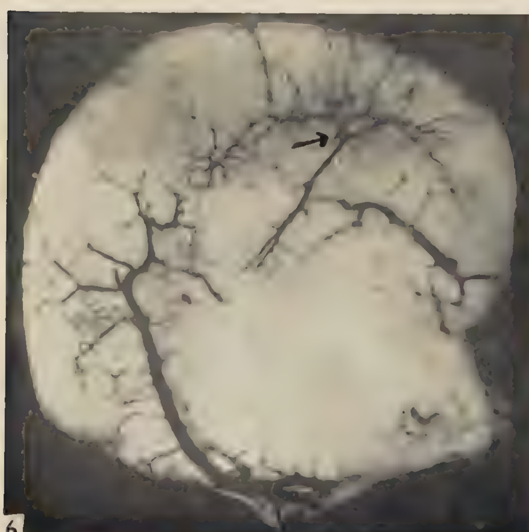

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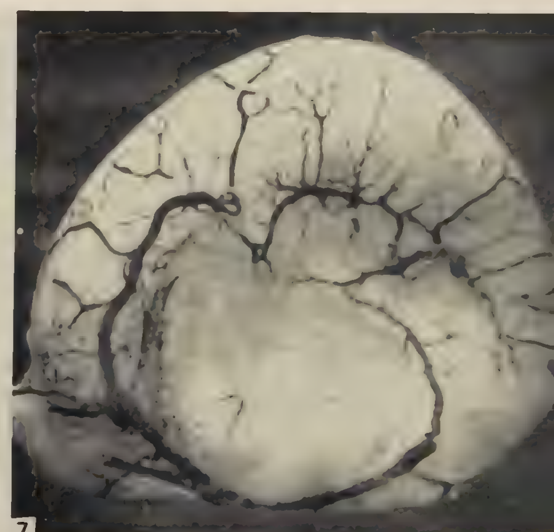

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MOFFAT—The development of the posterior cerebral artery

DEVELOPMENTAL CHANGES IN EPIDERMAL INNERVATION*

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INTRODUCTION

The mature nervous system is widely regarded as a highly stable organization. This view is no doubt based on the fact that, long before birth, the number and position of the cells of this system are already determined. The population of muscle fibres is also fixed at that time, and the number and disposition of their motor nerves appear to persist unchanged during the subsequent growth period.

The peripheral nerve terminals find themselves in a quite different milieu in the tissues of the integument. Here there is a continual change. Referring to the constant mitotic activity of the cells of stratified epithelia, Cowdry (1932) observed: 'Either the contact between the epithelial cells and nerve terminals is slowly shifting or else we have to do with a continual formation during the life of new terminals as outgrowths from larger nerve processes.' Weddell & Glees (1941) have proposed a solution to this problem. In the normal rabbit ear they have observed that a small number of the nerve fibres (from 1 to 5 %) showed signs of degeneration; regenerating fibres were also present. Degenerative changes were most common in the cutaneous nerve plexus. The number of degenerating fibres in the dorsum of the ear varied greatly from one animal to another. More recently the senior author has conducted another study of the innervation of the rabbit ear (Weddell & Pallie, 1955; Weddell, Pallie & Palmer, 1955; Weddell, Taylor & Williams, 1955). In this work, about 0·02 % of the nerve fibres in the rabbit ear showed signs of degeneration. The degenerating fibres were located in the main nerve bundles—none were seen in the cutaneous nerve plexus. The conflict with the earlier findings is not adverted to in these texts.

Dynamic changes in an amphibian peripheral nervous system have been described vividly by Speidel (1932). By a cinematographic technique he has been able to observe the behaviour of individual nerve fibres in the tail of the tadpole for periods of a month or longer. Neuronal growth takes place by extension of axons towards the epidermis and by subepidermal branching. Yet regressive features are part of the normal growth pattern: retraction of side-branches is common, and older branches are often eliminated by autotomy, i.e. by preterminal amputation (Speidel, 1940–41).

Thus Speidel has established that, in the tail of the larval amphibian, the total number of cutaneous nerve fibres increases with age, and that this increase is brought about by dichotomy. The literature does not reveal whether these observations hold good for all vertebrates. In mammals pleomorphism multiplies the problems

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† Holding an Ordinary B.M.A. Research Scholarship.

so that we may ask: During the growth period does the number of freely ending cutaneous nerves continually increase? Do hair follicles acquire a more profuse nerve supply? And do the encapsulated end-organs increase in number? It is with the first of these three questions, and with its implications, that this paper is concerned.

THE LITERATURE

We do not know whether the nerve supply of the mammalian epidermis increases throughout the growth period. The answer to this problem is not readily provided by human skin, as in man the majority of the nerve fibres from the cutaneous plexus end in the dermis; intra-epidermal fibres are scarce (Wollard, 1936). Tello (1932) has applied Cajal's silver methods to the human clitoris and found that the axons making contact with the basal layer of the epidermis are less numerous in the adult than in the young subject. It is an incomplete account in the present context as it does not reveal whether the total number of epidermal nerve fibres is affected by growth of the skin.

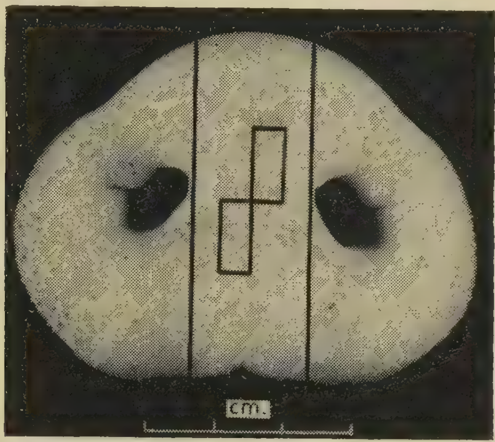
The skin is of a special kind in the nose of certain quadrupeds (pig, ox, cat, dog, rat, hedgehog, mole, etc.). The epidermis here is thickened and is deeply penetrated by nerve fibres. Its richness of innervation is without parallel, and has attracted the attention of many workers. Cauna (1959) has studied the influence of the multiplication and shedding of the epidermal cells of the cat's snout: he finds signs of neuronal death as well as death of epidermal cells. Nerve fragments, large and small, are cast off with the surface cells, a process which demands the continual elongation of the intra-epidermal axons. Cauna's appears to be the only study of the influence of *radial* epidermal growth: as already stated, the influence of *tangential* growth is not known.

MATERIAL AND METHODS

Histological methods suited to the problems in hand were first devised using the snout skin of some twenty animals. Fourteen healthy Large White pigs were then selected, at the following ages: 34 mm. embryos (2), 200 mm. embryos (2), 24 hr. after birth (1), 4 days (1), 2 weeks (1), 6 weeks (1), 16 weeks (1), 6 months (1), 1 year (1), 2 years (2), 4 years (1). The head of each animal was perfused with normal saline, followed by Richardson's (1958) sucrose-paraformalin. The embryos were perfused through the left ventricle of the heart; the other animals less than six weeks old were perfused *in vivo* through both common carotid arteries under intraperitoneal urethane anaesthesia. The older animals were killed in abattoirs by electrocution; their heads were promptly removed and tissue blocks taken for other purposes as required; bilateral carotid perfusion followed.

The area of the skin of the nasal septum was calculated. The septal skin was defined for this purpose as the area lying between vertical lines drawn along the medial margins of the nares (Text-fig. 1). In four pigs this area was estimated before the head was perfused and again 2 hr. later. There was no detectable difference between the first and second measurements: accordingly the area in the remaining animals was ascertained after perfusion. The skin of the septum was split by a sagittal incision in the mid-line, and divided into quadrants by a horizontal cut joining the centres of the nasal apertures. Blocks from opposite quadrants (e.g.

upper left and lower right) were taken and processed in pairs. One pair was fixed in formol-acetic-alcohol (Bodian, 1937) for 20–48 hr., dehydrated and embedded in paraffin. Serial sections at 15 μ were stained with protargol by a modification (FitzGerald, 1961) of the method of Davenport, McArthur & Bruesch (1939). The other pair was fixed for 3–5 weeks in sucrose-paraformalin; frozen sections were cut at 30 μ and every sixth section processed by a Gros–Bielschowsky method.



Text-fig. 1. Anterior view of pig's snout, 6 months after birth. The nasal septal skin is defined and the method of selection of tissue blocks is shown.

Quantitative estimates

Evans, Cowdry & Nielson (1943) showed that the shrinkage of biopsies of human skin, following formal fixation and paraffin embedding, tends to decrease with age. In pig material, therefore, the length of nine tissue blocks was measured before fixation in formol-acetic-alcohol, and again in the final wax bath following dehydration (Table 1). Correction for shrinkage was carried out where necessary.

All tissue blocks were between 3 and 5 mm. in diameter; they were sectioned on

Table 1. *Shrinkage of paraffin-embedded tissue blocks from the pig's snout. The degree of shrinkage at* 119 *and* 130 *days was not measured; from the Table the respective values were inferred at* 83 *and* 85 %

Days after service	Percentage of length
30	80
85	84
115	83
160	83
160	90
230	90
300	90
480	91
850	93
1600	94

either the medial or lateral surface, on the assumption that within this narrow zone there were no significant population differences in any one animal. In practice the area sampled by each method at each age was larger than the proper proportionate area—two strips some 4 mm. wide with a combined mean length of approximately 23 mm. ($24 \pm 1{\cdot}7$ mm.) in the paraffin-embedded material, $22 \pm 1{\cdot}3$ mm. in the other. The combined lengths are shown in Table 2 as a proportion of the full length.

Table 2. *Age of animals in relation to area and length of septal skin examined in paraffin sections. Areas and lengths are corrected for shrinkage*

Days after service	Area of estimate (mm.^{2})	% of septal area examined	% of septal length
30	2·1	50	100
30	2·1	50	100
85	5·6	18	100
85	5·6	18	100
116	9	7	100
119	8·6	4·3	100
130	9	3·3	100
160	9	2·2	100
230	9·2	1·3	40
300	9·45	0·76	44
480	7·3	0·35	21
850	9·35	0·42	30
850	9	0·38	32
1600	7·8	0·29	21

Calculations from paraffin sections

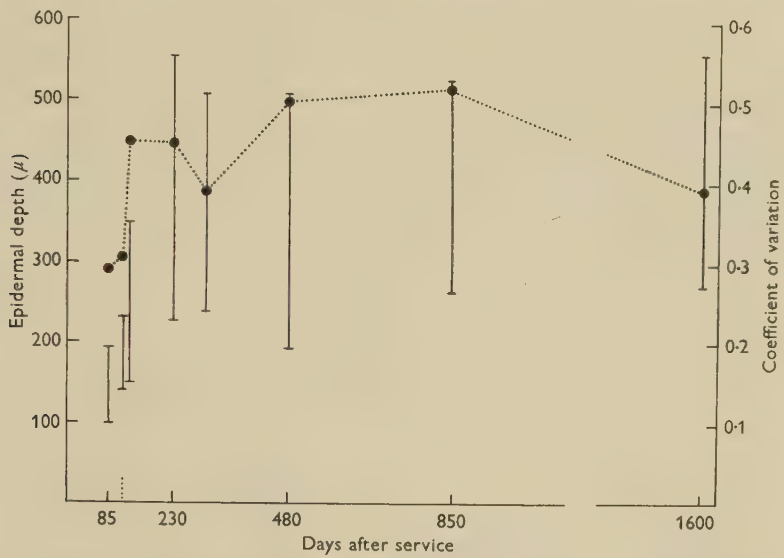
(i) *The number of nerve fibres entering the epidermis* (*fourteen animals*). At a magnification of 450, individual axons were examined at their point of entry from the dermis in every fourth section. Only those axons were counted which could be seen to penetrate between adjacent cells of the basal layer. Pl. 1, fig. 2, shows three such fibres entering from a dermal papilla. This photograph reveals also that it is only on the 'left' and 'right' faces of a sectioned dermal papilla that axons can be traced in continuity from lamina propria into epidermis. Because of the steepness of the epidermal ridges sections exposing their superior or inferior aspects were equally common. Fibres coursing upwards on these aspects were not counted, as their point of exit from the lamina propria could not be established with certainty (Pl. 1, fig. 1). The variation with age of the depth of the dermal papillae from the mean at that age (as estimated by the coefficient of variation of mean epidermal depth) did not appear likely to influence sensibly the number of fibres counted (Text-fig. 2). Accordingly, comparisons were drawn between the number of nerve fibres counted at various ages; it was for the purpose of such comparison that the estimates 'number per unit surface area' and 'total numbers' (the former value multiplied by the value for the surface area of the nasal septal skin) were made—the values obtained were not an assessment of the *actual* numbers in any tissue block.

(ii) *The number of subepidermal axon bifurcations* (*nine animals*). At a magnification of 450, the number of dividing axons within $250\,\mu$ of the basal layer of the epidermis (except those on hair follicles) was counted in every fourth section. Conclusions based on these counts were naturally tentative: many bifurcations were

masked by overlying fibres, many more were in all probability only apparent because of the passage of the microtome knife across the point of intersection of two fibres.

(iii) *The number of epidermal ridges (pegs).* These were counted at a magnification of 50 in every eighth section (twelve animals).

(iv) *The mean depth from stratum lucidum to epidermal basement membrane (six animals).* At a magnification of 100, twenty-five measurements were taken at intervals of 150–200 μ through each area, yielding fifty observations from each animal.



Text-fig. 2. Epidermal depth. Each vertical line represents the 95 % confidence limits of fifty measurements. The dotted line links the coefficients of variation of mean epidermal depth.

Calculations from frozen sections

(i) *The length of intra-epidermal nerve fibres.* In material from an animal of 6 weeks, the 'rectilinear' and actual lengths of 100 intra-epidermal nerve fibres were measured as follows. At a magnification of 1000 a camera lucida drawing of each fibre was made. The ratio determined was that between the length of a line joining the ends and that found by tracing the course of the fibre by means of an opisometer. The 100 fibres were made up of 50 successive fibres whose diameter was less than 1·5 μ and of 50 in excess of 2 μ.

(ii) *The number of fragmenting nerve fibres in the epidermis of the snout.* In each of nine animals, 200 successive intra-epidermal nerve fibres were examined; the percentage showing evidence of degeneration was recorded.

Other methods

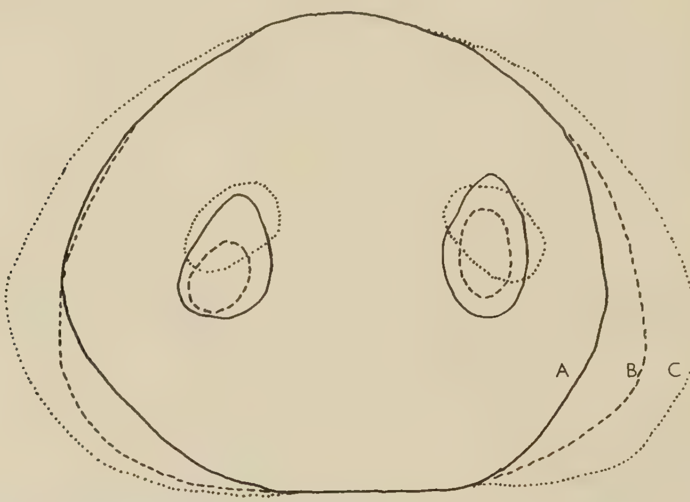
The cutaneous nerves were studied in paraffin sections (formol–acetic–alcohol fixation) by the methods of Romanes (1950) and Peters (1958); and in frozen sections (formol–calcium fixation) by Sudan Black B. The cellular structure of the epidermis was examined in paraffin sections following fixation in Fleming's fluid, and in

paraffin sections (Helly's fluid, formol–acetic–alcohol) stained with haematoxylin and eosin, Mallory's connective tissue stain and phosphotungstic acid haematoxylin. The epidermal keratin was examined in lightly impregnated Gros–Bielschowsky sections by plane polarized light and with the compound polarizer (MacConaill, 1957), which colours birefringent fibres according to their orientation in the visual field.

OBSERVATIONS

The gross anatomy

The skin of the pig's snout is supplied entirely by the infra-orbital nerve. This massive trunk breaks into a leash of nerve bundles on emerging from the infra-orbital foramen: one or two of these are joined by branches of the facial nerve, and twigs are given to the overlying skin and to the tissues of the upper lip. Without perceptible depletion the main branches proceed forwards to end in the skin of the anterior surface of the nose; the skin of the nasal septum is supplied by bundles which skirt the narial margins.



Text-fig. 3. Superimposed tracings of photographs of the pig's snout. A, 70 mm. embryo; B, 1 week, C, 2 years after birth.

The skin of the snout has vibrissae. They number about 350 and are more numerous in the lower part of the snout. The disposition of the follicles shows that growth of the skin of the nasal septum is interstitial, i.e. the new skin is formed between the follicles, not at the margins of the septum. For (*a*) the vibrissae are spaced farther apart in proportion to the increase of surface area of the septal skin, and (*b*) they always cover about 90 % of the skin surface, being absent from a narrow strip at the upper margin of the septum and from the skin immediately adjacent to the nares. The rates of vertical and transverse growth of the snout as a whole are unequal, that of the transverse diameter being greater; thus the snout becomes relatively broader as age advances (Text-fig. 3). The nasal septum on the other hand has practically equal rates of growth in both diameters.

Histology

The branches of the infra-orbital nerve run obliquely through the subcutaneous tissue to enter the skin; in the corium they communicate freely to form the dermal plexus from which the terminal nerve bundles emerge to cluster round the apices of the interpapillary ridges of the epidermis. The great majority of axons enter the epidermis; a minority of fibres apply themselves to the basal layer in the form of Merkel's discs; and a second minority turn aside under the epidermis to enter encapsulated end-organs.

The shape of a typical epidermal ridge is that of an inverted cone projecting into the dermis. Adjacent ridges usually project from a common cellular plateau: dermal papillae are therefore shallow as a rule although some—about one in ten—project deeply into the epidermis. The apices of these longer papillae reach the epidermis in the intervals between the bases of neighbouring epidermal ridges; here the stratum basale is separated from the stratum granulosum by only one or two prickle cells. Nerve fibres penetrate the tips or sides of the epidermal ridges, or the common cellular plateaux; but they are never seen to penetrate the shallow epidermis between the bases of adjacent ridges.

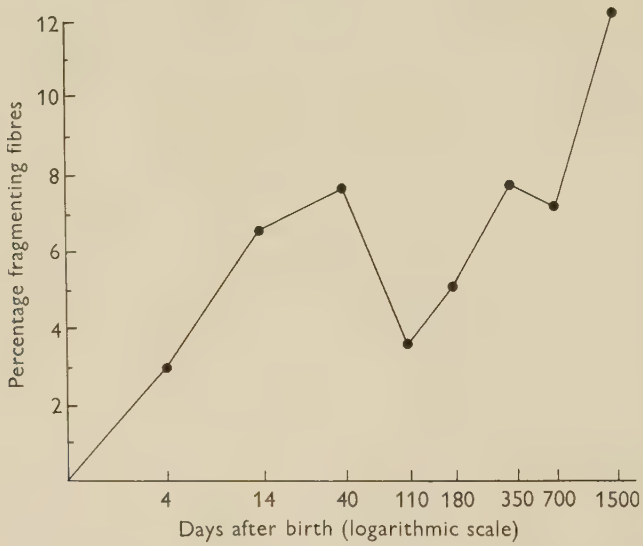
Within the epidermis the most striking feature is the variability in thickness of the nerve fibres. The finest visible axons penetrate only to the lower prickle cells, and do not divide. Fibres of medium and large calibre send branches to the upper prickle cells and to the cells of the stratum granulosum (Pl. 1, fig. 3; Pl. 2, fig. 8): on these axons terminal vesicles are the rule. Smaller swellings are commonly found along their length, particularly where they show a change of direction, as noted by Kadanoff (1928); terminal varicosities are often multiple (Pl. 1, fig. 4; Pl. 2, fig. 7) and some of these have seemingly separated from their parent nerve fibres. The vesicles, apparently isolated, show an affinity for silver; they are common among the outer granular cells and may be found even in the stratum corneum. Intact axis cylinders are rarely seen among the outer granular cells, and they never penetrate the horny layer. Schwann sheaths appear to invest epidermal neurones only as far as their point of entry. Whitear (1960) has demonstrated a similar arrangement in the case of axons which enter the corneal epithelium.

The thickest fibres stain intensely with silver and stand out from their background. They exhibit irregularities of contour and they do not branch as freely as the smaller fibres; their tips are surmounted by groups of vesicles, frequently without any connecting axoplasmic threads. Many of these thick nerve fibres are broken into short or long segments (Pl. 2, fig. 10). In suitable preparations it is clear that the interruption is not due simply to the plane of section; under oil-immersion the adjacent ends may be seen to lie free in the middle focal plane. The appearances do strongly suggest that these nerve fibres are undergoing fragmentation, for the segments are irregular in outline and often stain in a patchy manner with silver, and at times a slender thread of pale-staining axoplasm is seen to join the central to the peripheral stump. Again, the faint 'image' of a nerve segment may be seen in the upper part of the epidermis. Axonal degeneration does not extend below the epidermis. The fate of the severed central stump is unknown: it may resume the growth cycle. Collateral branching from the central stump has not been seen.

Myelin staining with silver characterizes the subepidermal portion of the thickest fibres (Pl. 2, fig. 9) including those showing evidence of fragmentation. It may be traced for distances of 20–100μ to the point where the fibres become lost among large nerve bundles.

No degenerate fibres have been observed in two 200 mm. embryos or in four new-born animals. Percentage estimates (numbers of fragmenting axons per 100 successive nerve fibres) indicate that terminal degeneration, although variable in its incidence, tends to increase with advance in age (Text-fig. 4).

The changes outlined above afford some account of the influence on free nerve endings of a continuously changing environment. Yet it is not correct to consider the nerve terminal as merely drawing out an axoplasmic thread while following the outward drift of the surrounding cells. This drift must tend to maintain the



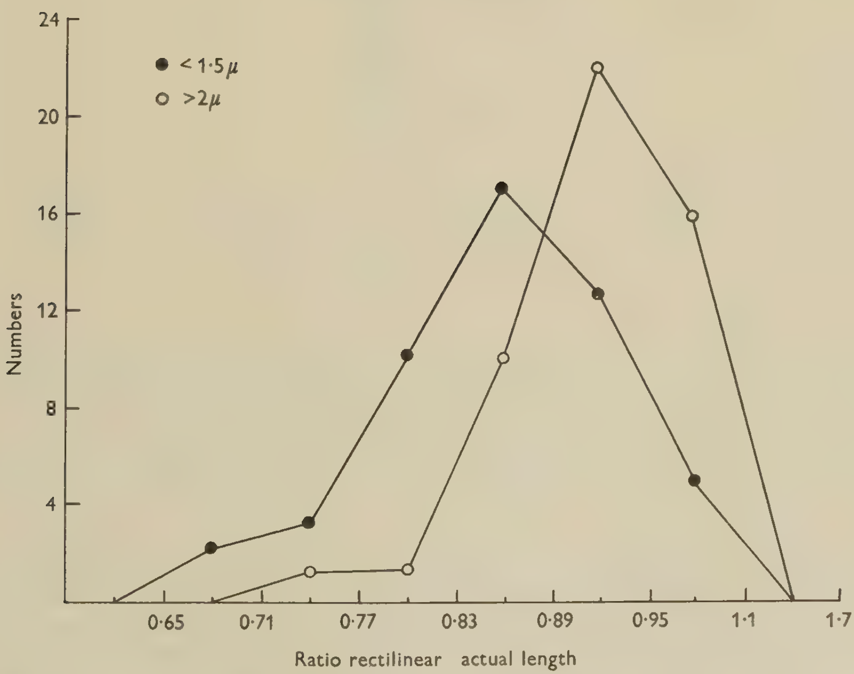
Text-fig. 4. Percentage of fragmenting intra-epidermal nerve fibres relative to age.

axons in correct alignment as Cauna (1959) suggests; but there is also evidence of some less obvious factor. In Text-fig. 5 the intra-epithelial course of six axons is reproduced. Each is tortuous; yet the terminal parts of all are in the same vertical plane. Looped intra-epidermal fibres have been observed in young animals on at least twenty occasions. Looping is merely a striking example of the tendency of fibres to turn aside or even reverse direction before proceeding once more towards the stratum granulosum. Patterns such as those shown in Text-fig. 5 might conceivably have been brought about by displacement, due to irregular movements of cells through the prickle layer. The effects of distortion produced in this way should be more apparent among fibres long subject to such an influence. Assuming that all the coarse fibres are older than some of the fine, a larger number of the coarse fibres may be expected to be tortuous. The findings shown in Text-fig. 6 indicate that this is not so. In this figure the ratio of over-all (rectilinear) length to actual length of

100 axons is given: the 50 slender axons are significantly more curved than the 50 broad ones ($P < 0{\cdot}01$).

The conclusion follows that the irregular disposition of thick nerve fibres represents the earlier pattern of those which are now coarse. In so far as the progress of a pioneering nerve tip is determined by the structural configuration of its environ-

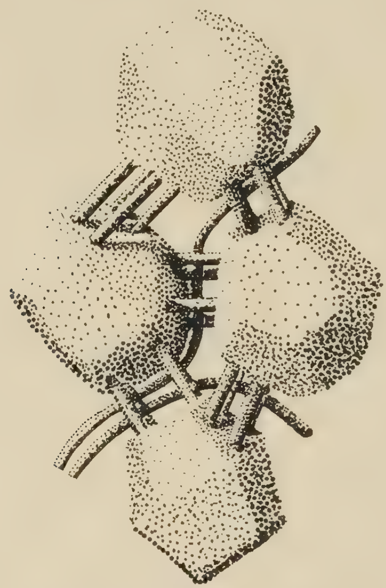
Text-fig. 5. Camera lucida drawings of six axons in the snout epidermis (frozen sections) 6 weeks after birth.



Text-fig. 6. Frequency polygon showing the ratio of rectilinear (overall) to actual length (R:A ratio) of 100 successive intra-epidermal axons in frozen sections 6 weeks after birth.

ment—i.e. inasmuch as intra-epithelial neuronal growth is stereotropic—its progress will be along the path of least resistance. This pathway will guide it between adjacent epidermal cells and along the keratin scaffolding of the epidermis.

Due to mutual pressure, the columnar basal cells tend to take the form of hexagonal cylinders (Pettigrew, 1908); the prickle cells are also polyhedral, presenting six surfaces in each plane of section, and the intercellular bridges which project from each surface radiate in all directions. The stratum Malphighii of the pig's snout is rich in intercellular bridges. In paraffin sections at 6 months the bridges are found to be about $0{\cdot}4\,\mu$ in thickness and are spaced $0{\cdot}4\,\mu$ apart (phosphotungstic

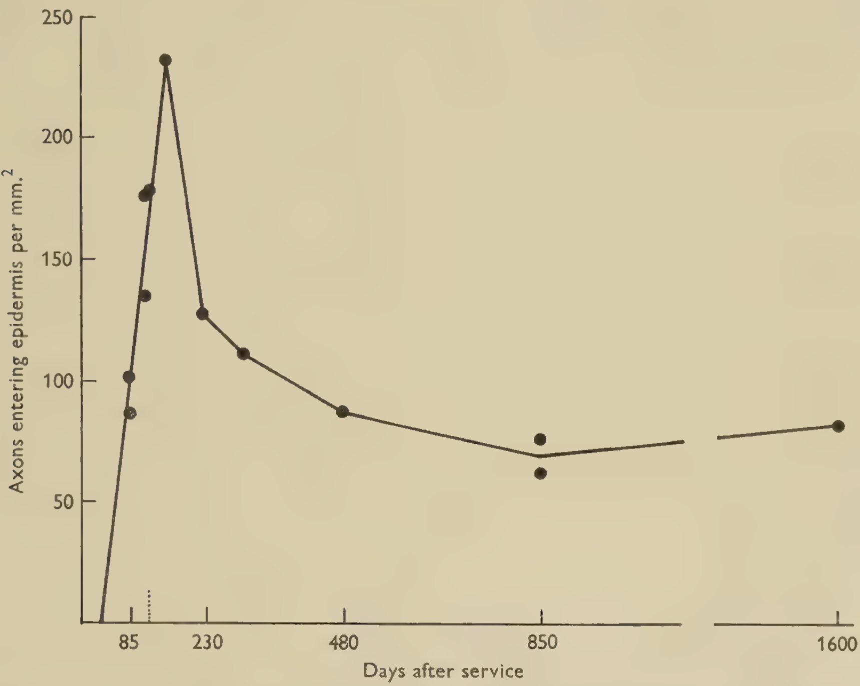


Text-fig. 7. A diagram to show the relationship of axons to prickle cells. Two axons are shown passing between intercellular bridges; the upper axon has been guided towards the surface by the cell contours but the lower axon, travelling from the same point, has been deflected downwards.

acid haematoxylin). Nerve tips which have penetrated the basement membrane will be guided to the spinous layer along a path conforming to the shape of the basal cells. In penetrating the spinous layer they must pass through the holes in the intercellular latticework. Text-fig. 7 is a diagram illustrating two nerve fibres in this part of their course; it shows that, on our present knowledge, a cell arrangement of this type cannot establish a preferential pathway for insinuating nerve fibres.

The keratin skeleton *is* preferentially arranged in the spinous layer. Under polarized light its fibres are weakly birefringent and are often curved; they intersect in three main directions, one vertical and two oblique. In the absence of a definable horizontal set, the resultant orientation is vertical. In this respect the keratin pattern conforms with that of the skin in other orders (Matoltsy, 1958). In the stratum granulosum and among the outer three or four rows of prickle cells, the

keratin, again weakly birefringent, conforms with the horizontal, long axes of the cells; here both cells and keratin would appear to militate against the passage of axons. The greater number of epidermal nerve fibres do not in fact reach this level. Two fibres which have penetrated among the granular cells are shown in Pl. 2, figs. 5 and 6. Terminal undulation is the rule among fibres which gain the granular layer: nevertheless, their overall orientation remains vertical. A minority of terminals do not undulate; and a second minority conforms with its background in taking a horizontal course.



Text-fig. 8. The numbers of visible axons entering the epidermis per mm.² surface area (corrected). The average age at birth is indicated by the short dotted line.

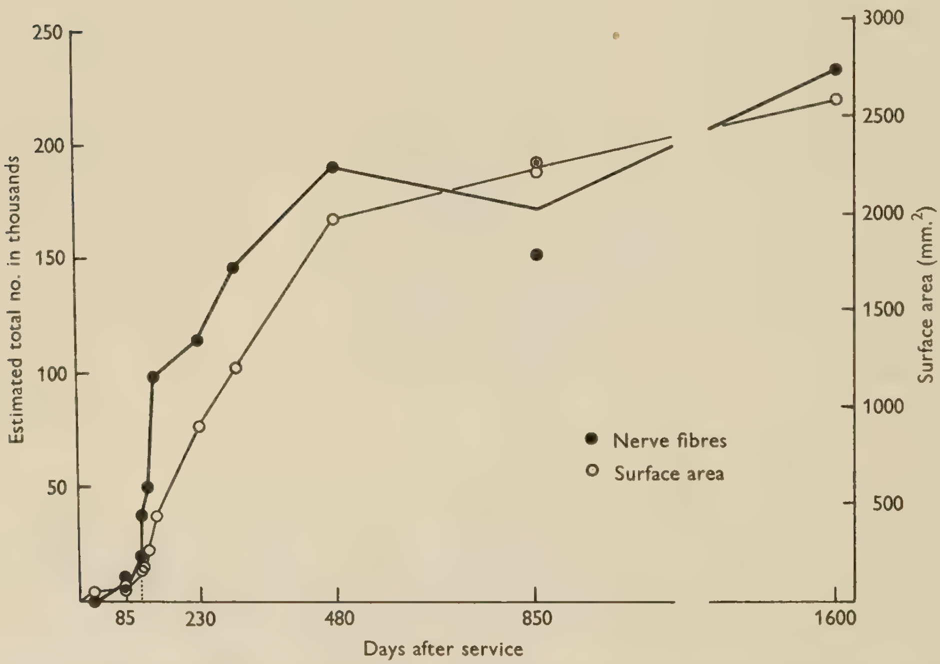
These considerations of epidermal architecture do not provide a complete explanation for the arrangement of intra-epidermal axons. The cells of the prickle layer seem to be arranged indifferently, while the granular cells evidently do not favour vertical growth: the keratin skeleton may provide a favourable foothold below but does not do so above. The path taken by atypical fibres suggests that their environment does not determine the orientation and the re-orientation of intra-epidermal nerves: it suggests rather, the notion that the upgrowth of nerve fibres proceeds in spite of a tendency of the physical environment to obstruct and to deflect. The sinuous course of young fibres in the spinous layer, the frequent coiling of fibres and the tortuosity of terminals penetrating the granular layer—each observation supports the contention that in the epidermis the growth of neurones is controlled by an agency other than those hitherto considered.

Nerve fibre populations. The number of optically visible fibres entering the

epidermis per unit area was maximal in young animals. It rose steeply from zero at 30 days after conception, to reach a peak value of 234 6 weeks after birth (Text-fig. 8). A gradual decline followed, the number falling to half the 6-week figure 6 months after birth. The 2-year and 4-year values are similar to that of 1 year. There was

Table 3. *Numbers of epidermal axons per mm.² (uncorrected) in the uppermost, middle and lowermost quarters of the nasal septal skin*

Age in days ...	85	85	115	119	130	160	1600	Mean
Upper quarter	125	110	177	180	187	270	90	163
Middle quarter	114	98	166	245	217	275	95	173
Lower quarter	160	140	190	236	250	280	118	196
Mean	133	116	178	220	218	275	101	

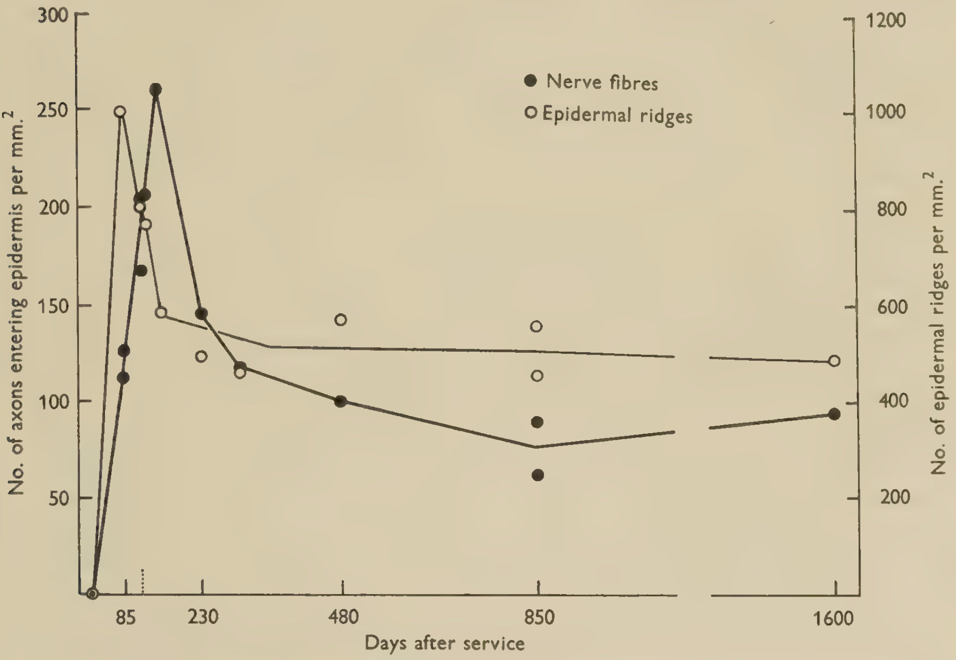


Text-fig. 9. Estimated total numbers of visible nerve fibres entering the epidermis (corrected) compared with the surface area of the septal skin at post mortem.

the possibility that the relatively high fibre counts might have been due to the inclusion, near the upper and lower ends of the septum, of intra-epidermal axons in relatively high concentration; these would have been omitted from the later tissue blocks which tend to sample the centre of the septum (Table 1). In order to test this possibility, the material from seven animals was examined further. The sections from upper and lower blocks were brought together, and the septum was considered to be divided into five parts. The even numbered parts, each one-eighth of the total length, were excluded. From the 4-year old animal tissue blocks from the uppermost and lowermost quarters of the septum were retained; these were sectioned and examined. Table 3 shows the result of the test; and it can be seen that the mean

concentration was not least in the middle of the septum but was intermediate between that at top and bottom. Prescinding from technical and sampling errors, the data in Text-fig. 8 are therefore valid as an index of nerve fibre populations per unit area; the assessment of total values in the manner described is also valid.

Text-fig. 9 shows total values. The estimated number (corrected for shrinkage) of visible nerve fibres entering the epidermis is zero at 30 days, rising to 17,400 at birth. In the immediate postnatal period the curve shows a sharp rise: at 3·5 months the total number is already seven times the neonatal value. In all the animals under 1 year the increment of nerve fibres exceed the increment of surface area; after 1 year the increment of nerve fibres falls considerably, the 4-year value being only



Text-fig. 10. Concentrations (uncorrected) of epidermal axons and of epidermal ridges.

1·2 times the 1-year value. The limit estimates for the postnatal period indicate the overall increase in the quantity of skin in the nasal septum after birth, and in the quantity of visible epidermal fibres: the surface area at 4 years is 20·5 times greater than on the first postnatal day; the number of nerve fibres is fourteen times greater.

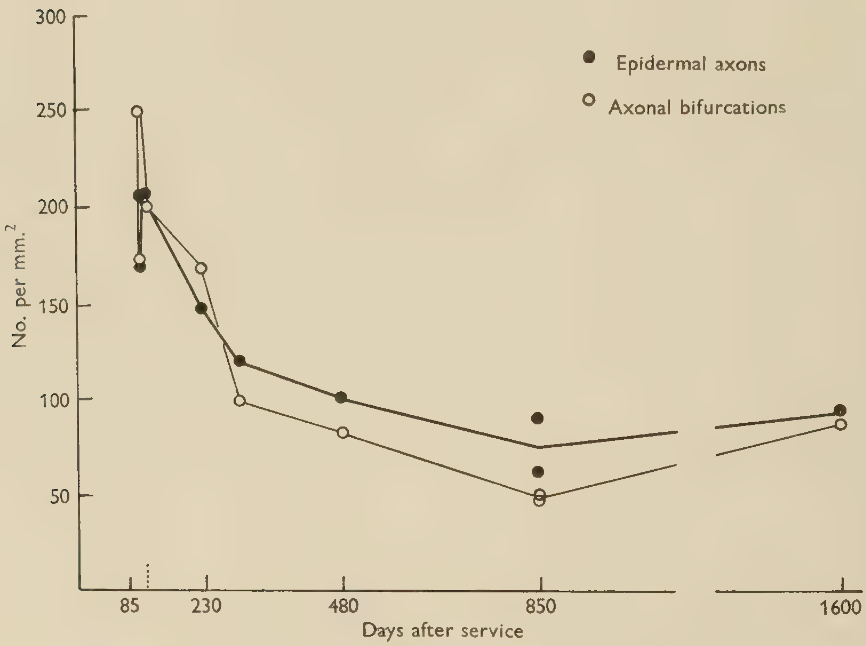
The epidermal ridges are most closely packed in a 200 mm. embryo (Text-fig. 10). In the material from the early postnatal period their concentration (number per unit surface area) falls sharply to reach half that of the embryo at 3·5 months. A relatively steady state is observed in the later specimens, whose concentrations lie within $\pm 8\%$ of the 3·5 month value.

Correlation of the number of epidermal ridges with that of intra-epidermal nerve has been carried out in two ways. First, the coefficient of linear correlation has been determined from the data in Text-fig. 10. The correlation coefficient (r)—in the postnatal specimens—is 0·67. This is probably significant ($P = 0{\cdot}025$). Secondly,

the material listed in Table 3 has been examined further: the numbers of epidermal ridges in the odd-number quarters of the nasal septa have been determined (Table 4). The linear correlation coefficient of 0·76 is highly significant ($P < 0·001$).

Table 4. *The numbers of epidermal ridges per mm.² (uncorrected) in the uppermost, middle and lowermost quarters of the nasal septal skin*

Age in days ...	90	90	116	119	130	160	1600	Mean
Upper quarter	1080	1080	689	793	680	410	499	759
Middle quarter	1020	1080	915	892	706	603	501	835
Lower quarter	930	1050	696	916	750	775	515	814
Mean	1010	1070	767	867	712	596	505	



Text-fig. 11. Concentrations (uncorrected) of epidermal axons and of dermal axonal bifurcations.

Subepidermal nerve fibres are already numerous in the 34 mm. embryo. The interval between the epidermis and the nasal septal cartilage is intersected by nerve bundles which seldom communicate but which branch freely under the epithelium. In the nerve bundles no structural detail is discernible; the fibres are matted together and none can be traced into the epidermis. In the 200 mm. embryo the nerves are both coarse and fine; among them lie pale-staining groups of fibres which are apparently extremely small. Peters & Muir (1959) have shown in rat embryos of comparable age that the aggregates of pale-staining fibres are made up of hundreds of minute axons (less than 0·2 μ in diameter): only in full-term animals do the nerve fibres stain individually with silver. The number of manifestly discrete nerve fibres in the dermis is largest in new-born animals. In sections from the new-born there is

a striking disproportion between the relatively small number of intra-epidermal axons and the number coursing in large bundles towards the epidermal pegs. This appearance was at first thought to be due to inadequate technique; however, the absence of this phenomenon in more mature material suggests that the appearances were reliable. Pl. 2, fig. 11, illustrates the phenomenon; here nerve fibres were massed round the apex of an epidermal peg, yet none could be traced into it. This evident disparity between dermal and epidermal nerve fibre concentrations was seen in six animals less than a week old; at 2 weeks, and afterwards, the density of nerve fibres in the dermis was less and the disparity seldom apparent.

Axonal bifurcations are seen in profusion in the dermis. The size, position and direction of almost all the daughter fibres (other than those on hair follicles) identifies them as epidermal. Within a distance of $250\,\mu$ of the epidermis nerve fibres to blood vessels were seldom encountered. In Text-fig. 11 the postnatal densities of dermal axonal bifurcations are compared with those of epidermal nerve fibres in the same material. Despite the difficulty of making accurate counts of axonal bifurcations, a highly significant correlation with epidermal axons has resulted ($r = 0{\cdot}99$, $P < 0{\cdot}0001$).

DISCUSSION

It cannot be asserted that *all* the intra-epidermal nerve fibres are displayed by the methods employed. The study of comparable material with the electron-microscope is a prerequisite for such an assertion: the mature central nervous system contains nerve fibres visible only by means of the electron microscope (Fernandez-Moran, 1954), and the same may be true in the peripheral. It may be that the epidermis is invaded by axons which, for a time at least, are invisible by light microscopy. In the absence of further information, the quantitative findings are considered to be accurate from the time of appearance of axons demonstrable with the light microscope.

In the epidermis of the pig's snout there is evidence of the growth and decay of nerve fibres, with ever-increasing numbers of axons contributing to an expanding sensory field. The finest fibres pursue a relatively tortuous course; the thicker fibres are straighter and are usually capped by axoplasmic vesicles. Precisely similar vesicles are found beyond the tips of some coarse fibres and in line with them. The appearances suggest that axoplasmic droplets accumulate at the tips of larger nerve fibres and are then shed. Whether all intra-epidermal axons enlarge as they mature is not known; on the assumption, made previously, that the coarse fibres are older than some at least of the fine ones, their relative straightness may be brought about by the outward (radial) drift of epidermal cells.

In the series under observation, there is a progressive increase with age in the number of nerve fibres entering the epidermis. Since in the postnatal series this number is directly related to the number of dermal axonal bifurcations, the fibres entering the epidermis after birth must have been produced mainly or entirely by the sprouting of collaterals from the dermal plexus. The dermis of the growing snout thus provides a neuronal pool from which the epidermis can acquire more fibres. Some light is shed on the possible mechanism of recruitment by experiments which have influenced the behaviour of peripheral nerve fibres.

Edds (1950) interfered with the serratus anterior muscle of the rabbit by cutting the contribution from the sixth cervical nerve. In partially denervated segments the residual intact motor fibres give off collaterals which invade the adjacent nerve tubes; proceeding to denervated muscle fibres, they arborized to form new motor end-plates. In the same year Hoffman reported preterminal sprouting of motor axons following partial denervation of the leg muscles of the rat; again, the new axons reached the denervated muscle via nerve tubes. Each worker concluded that the formation of collaterals was induced by the degeneration of adjacent nerve fibres, whether the induction be considered due to proliferation of Schwann cells (Edds) or disintegration of myelin (Hoffman). Van Harreveld (1952) confirmed these histological observations by means of a similar experiment on the rat's sartorius.

Sensory nerve terminals also react to partial denervation. In tadpoles, Speidel (1932) found that a denervated area of skin is invaded by nerve fibres independently of the Schwann cells of the degenerating nerve. Weddell, Guttmann & Guttmann (1941) recorded axon sprouting in response to partial denervation of the skin of the rabbit's leg. The nerve tubes of the denervated zone were invaded by sprouts from intact nerve fibres nearby.

Sprouting of axons under experimental conditions has been recently discovered in the autonomic nervous system. Murray & Thompson (1956, 1957) transected 90 % of the preganglionic nerve fibres to the superior cervical sympathetic ganglion of the cat: from the intact fibres—and especially from their terminal ramifications—fine collaterals arose and came into apposition with most of the cells of the ganglion.

The independent experiments of Aitken (1950) and of Hoffman (1950) are of special interest. Each worker used a leg muscle of the rabbit: having severed its nerve supply he implanted a neighbouring motor trunk into the muscle at some distance from the motor hilum. The new nerve branched freely and formed motor end-plates in new positions on the muscle fibres. The course of the branches was independent of the Schwann tubes of the proper motor nerve. When the proper motor nerve was left intact the implanted axons extended in the long axis of the muscle fibres without branching; they formed end-plates only where the muscle was injured at the point of insertion (Aitken). These authors appear to suggest that sprouting is induced, not by neuronal degeneration, but by *denervated muscle fibres*.

The present work has shown that the sprouting of collaterals from sensory nerve fibres is a feature of normal postnatal development. There is nothing to suggest that neuronal degeneration plays any part in the induction of sprouting: the fragmentation of nerve endings is confined to the epidermis and shows no quantitative temporal relationship to axon recruitment. The positive correlation between the changing populations of epidermal axons and of epidermal ridges (through which they enter) is manifestly compatible with a causal relationship. In each of the experiments referred to above a state of partial denervation precedes the sprouting process. In growing snout skin a fundamentally similar state could be brought about as follows. Tangential expansion leads to a continuous increase in the number of epidermal ridges; by a dispersion of existing intra-epidermal axons, tangential expansion may therefore lead to a state of relative *interstitial* epidermal denervation. It is now

suggested that this interstitial denervation is the stimulus for the continual increase in the number of epidermal nerve fibres.

The histological features do not reveal the immediate cause of sprouting in the dermal plexus, nor do they show how axonal branches are guided to and through the epidermis. Either physical or chemical factors must be responsible. Weiss (1934, 1936, 1941) has stated that stereotropism (the orientation of growing nerve fibres by the physical structure of surrounding tissues) may be sufficient to account for the grouping of axons into bundles and for the construction of nerve plexuses. There is no reason to suppose that chemical factors determine the organization of the infra-orbital nerve or of the dermal plexus in the snout: in the 34 mm. embryo the infra-orbital is already a massive trunk and its terminal plexus has appeared, yet its fibres have not made visible connexions with the epidermis of the snout. It does seem unlikely, however, that the later formation and proliferation of epidermal ridges so influence the 'untrastructure' (Weiss, 1950) of the dermis that new axons develop, not merely at the tips, but from the sides of the dermal axons. Cajal (1919) has supposed that the neural invasion of epithelia (including that of snout skin) in the embryo is determined by neurotropism—the attraction of growing fibres by an unknown chemical substance. Abnormal epidermis may indeed influence profoundly the density and orientation of subepithelial axons. Julius (1926) has found that some weeks after painting coal tar on the hairy skin of mice, increased cellular activity is shown by thickening of the stratum corneum. Simultaneously, sensory nerve endings are seen to proliferate vigorously, especially round the hair follicles (hyperneury). The new nerve branches in the dermis do not penetrate the overlying epithelium but turn back to end within the dermis. Ludford (1930) has confirmed these findings.

Although a linear correlation has been shown to exist between the growth in number of epidermal ridges and of epidermal nerve fibres, this relationship is modified by a time factor. The observed sequence is as follows. Epidermal ridges are formed in greatest numbers in the foetus; a month later (at birth) the concentration of nerve fibres beneath the ridges reaches its peak; in the following weeks the number of fibres penetrating the ridges is at its maximum. If there is in fact a causal relationship, then the neural response to the growth of epidermal ridges is a delayed one.

No positive information has been obtained about the mechanism by which intra-epidermal axons are aligned. It has been seen that neither the architecture of the epidermis nor the radial movement of its cells appear to offer a sufficient basis of explanation. It may be again that chemical rather than purely physical factors are operative: chemical diffusion of a kind that excites collateral sprouting may also guide the nerve branches towards the skin surface. A suitable gradient of chemical attraction could be established if the attracting substance is liberated by the most superficial of the epidermal cells. Alternatively, a gradient could be established if the attracting substance, liberated by newly formed epithelium, is neutralized from below by a complementary factor set free by upgrowing nerve fibres.

Tangential growth is associated with an ever-increasing total population of nerve fibres: radial growth is associated with the continual process of axonal fragmentation and renewal. No evidence has been found to show that radial growth *per se* is responsible for the fragmentation of axons. The findings are rather to be interpreted

as indicative of a process of ageing and consequent decay of intra-epidermal nerve endings; the effect of radial migration is probably only to remove the separated axoplasm, just as has been observed in the case of the varicosities which succeed one another at the tips of healthy, mature nerve terminals among the cells of the stratum granulosum. Fragmentation of nerve branches may be the result of the passage into these branches of a diminishing proportion of nutrient material as more and yet more axoplasm is diverted into fresh outgrowths. This may be a sufficient explanation of the observed tendency for the incidence of fragmentation to increase with age.

SUMMARY

1. The innervation of the epidermis of the pig's snout was studied in fourteen animals ranging from 30 to 1600 days after service. Histological observation was followed in each animal by quantitative estimates of degenerating intra-epidermal axons, of axons entering the epidermis, of subepidermal axonal bifurcations and of epidermal ridges.

2. Enlargement of intra-epidermal axons was accompanied by an increase in length and a decrease in tortuosity, and was followed by degeneration. Degenerating fibres first appeared 2 weeks after birth, their proportion tending to increase with further advance in age.

3. Neither the cyto-architecture of the epidermis nor its keratin skeleton appeared to provide a sufficient basis of explanation for the characteristically vertical orientation of intra-epidermal nerve fibres.

4. The concentration of nerve fibres entering the epidermis of the nasal septum rose steeply from 0 at 30 days to reach a maximum at 160 days; a more gradual decline followed. The estimated total numbers entering the epidermis indicated that a 20-fold postnatal increase in the surface area of the septal skin was accompanied by a 14-fold increase in the number of epidermal axons.

5. The postnatal increase in epidermal nerve fibre populations was explainable by the branching of axons within 250μ of the epidermal basement membrane.

6. There was a positive linear correlation between the numbers of epidermal axons in the uppermost, middle and lowermost quarters of the nasal septal skin and the respective numbers of epidermal ridges.

7. It is concluded that the growing epidermis of the pig's snout controls its own nerve supply. It is suggested that the sprouting of new epidermal axons is brought about by a state of interstitial denervation of the epidermis, comparable in its nature to that of partial experimental denervation. The observed fragmentation of intra-epithelial axons may be the result of the diversion of the more proximal axoplasm into fresh neural outgrowths.

Part of this work was carried out in the Department of Anatomy, St Thomas's Hospital Medical School, and I should like to express my gratitude to Prof. D. V. Davies for the facilities provided. I am indebted also to Prof. M. A. MacConaill for helpful discussions and for his criticisms of the manuscript.

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EXPLANATION OF PLATES

Plate 1

All figures are from paraffin sections of snout skin at 15μ, stained with protargol.

Fig. 1. 160 days. Medium and large intra-epidermal axons. Some fibres (bottom centre) are seen to enter from a dermal papilla; the points of entry of the remaining fibres are not seen.

Fig. 2. 160 days. Three axons entering the epidermis (arrows) from a dermal papilla.

Fig. 3. 119 days. Axon of medium calibre branching among the upper cells of the prickle layer.

Fig. 4. A fibre surmounted by axoplasmic vesicles (arrows) which extend into the stratum granulosum.

Plate 2

All figures are from frozen sections of snout skin at $30–40\mu$, stained by a Gros–Bielschowsky method.

Fig. 5. 1600 days. Two intra-epidermal axons.

Fig. 6. The same field in plane polarized light. The arrows in Figs. 5 and 6 point to an axoplasmic vesicle in the stratum lucidum.

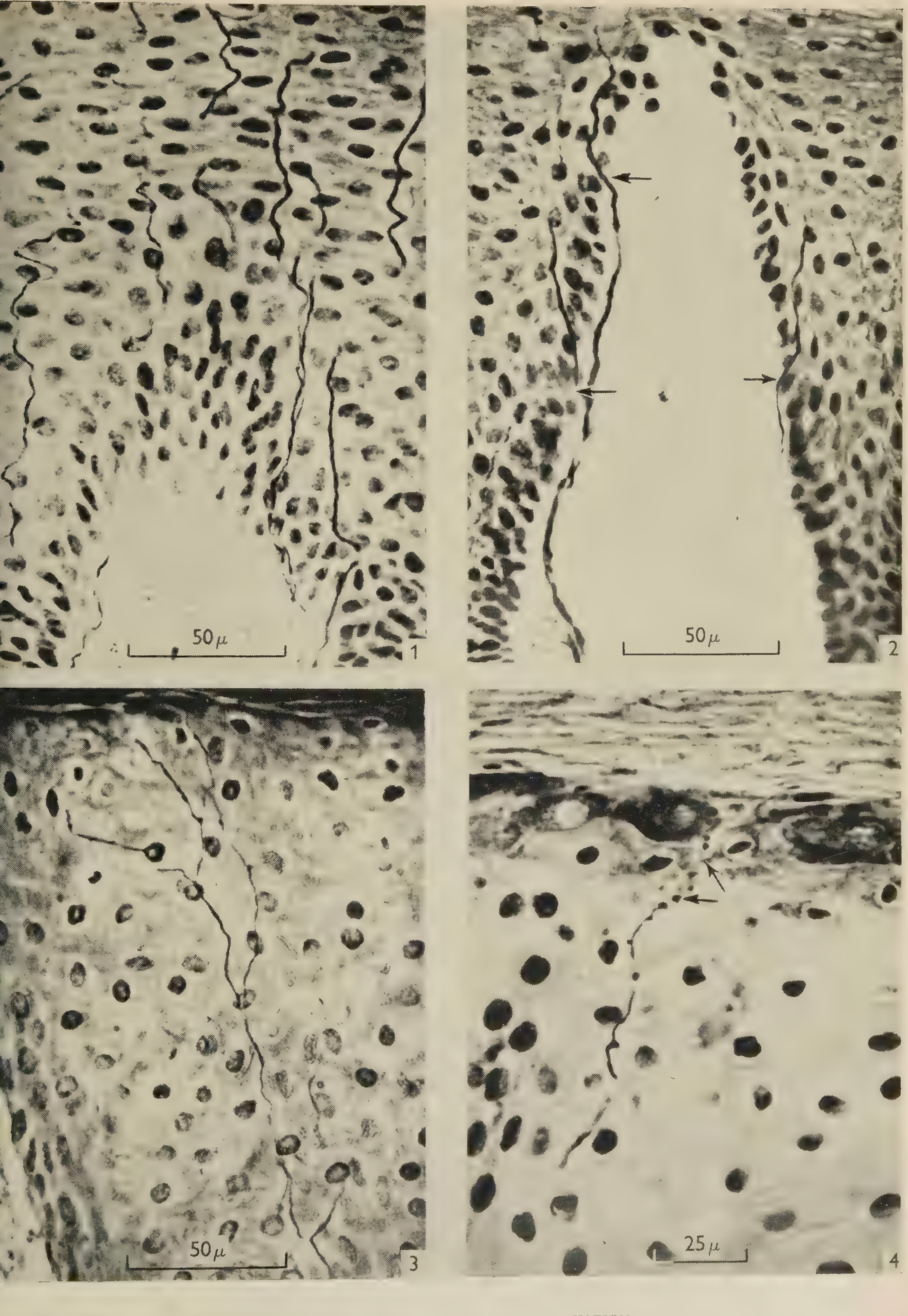
Fig. 7. 230 days. Terminal axonal varicosities in the (unstained) stratum granulosum.

Fig. 8. 160 days. A coarse fibre, branching among the upper prickle cells. The entire length of this fibre is intra-epidermal.

Fig. 9. 160 days. The lower part of this coarse fibre occupies a dermal papilla; it shows myelin staining (arrow).

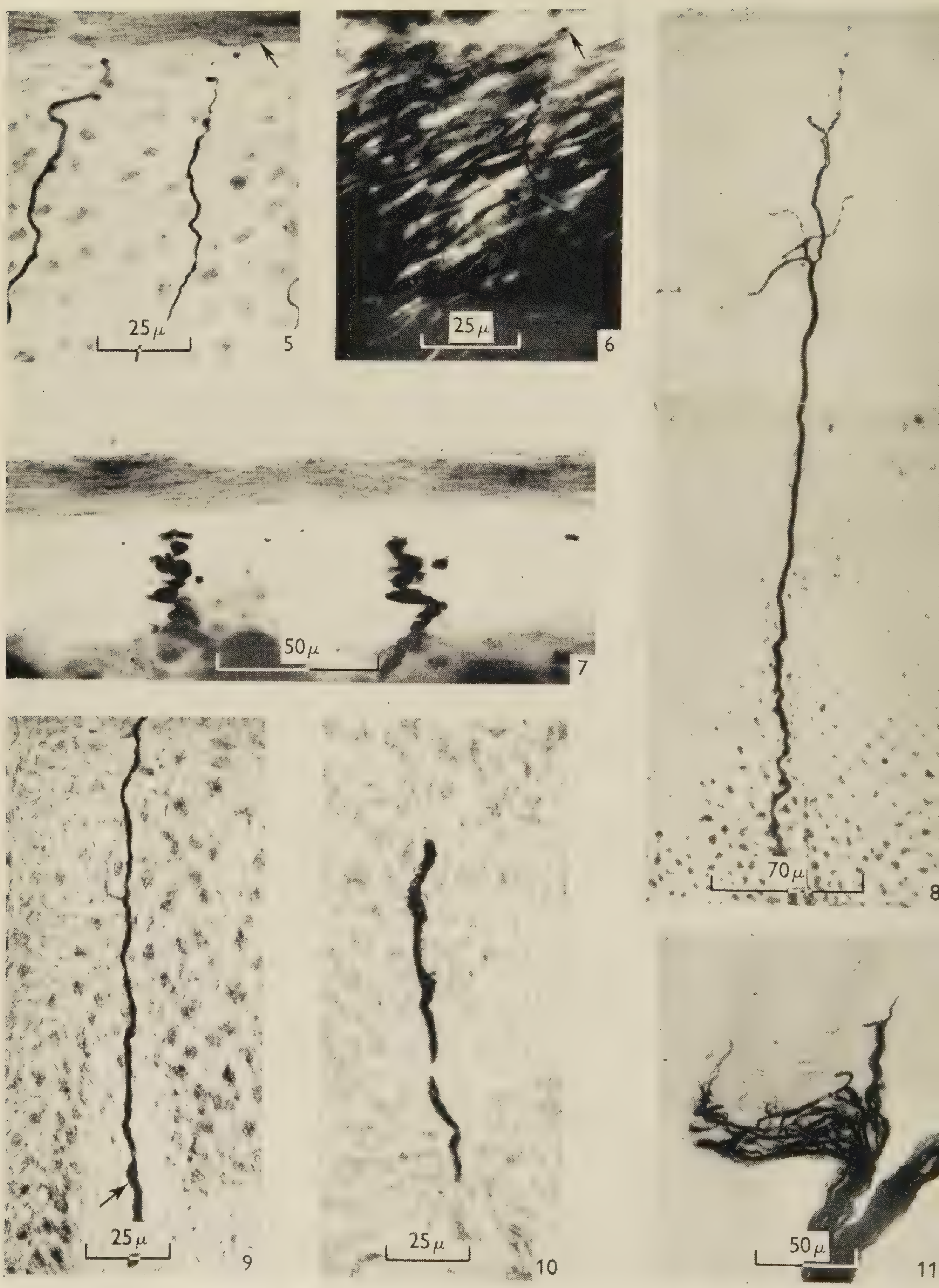
Fig. 10. 130 days. Fragmenting intra-epidermal axon; the upper segment is markedly irregular in outline.

Fig. 11. 160 days. Dermal axons clustered round the apex of an epidermal peg.



FITZGERALD—DEVELOPMENTAL CHANGES IN EPIDERMAL INNERVATION

(*Facing p.* 514)



FITZGERALD—Developmental changes in epidermal innervation

FIBRE DEGENERATION FOLLOWING LESIONS OF THE AMYGDALOID COMPLEX IN THE MONKEY

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Of the amygdaloid projection pathways the stria terminalis has traditionally received strong emphasis in anatomical studies, and less attention has customarily been given other fibre systems originating in the amygdaloid complex. It is, however, known that the amygdala is connected with other basal telencephalic structures and with the diencephalon by a massive ventral fibre system which spreads forward and medially through the region underneath the lentiform nucleus. This fibre system appears to have been recognized first by Johnston (1923), who considered it to be an amygdalofugal component of his 'longitudinal association bundle'. Johnston limited his account of this projection system to the statement that such fibres beneath the globus pallidus join 'the general system of precommissural fibres passing up through the parolfactory area'. Later workers, on the basis of experimental-anatomical observations in the cat, described distributions of the ventral amygdalofugal fibre system to the preoptic region and hypothalamus (Lammers & Lohman, 1957; Hall, 1960), the bed nucleus of the stria terminalis (Fox, 1943), the caudate nucleus and the subcallosal gyrus (Lammers & Lohman, 1957).

In Marchi experiments in the monkey, Fox (1949) made the interesting observation that fibres of apparently the same general category join the inferior thalamic peduncle and terminate in the dorso-medial nucleus of the thalamus. In a recent study by the aid of the Nauta–Gygax silver technique the existence of a direct amygdalo-thalamic projection in the monkey was confirmed, whereas other components of the ventral amygdalofugal fibre system were traced to the substantia innominata, the lateral preoptic and hypothalamic regions, the basal septal region, olfactory tubercle, and rostral limbic cortex (Nauta & Valenstein, 1958). These findings have hitherto been published only in the form of an abstract. The present paper will serve to present a more detailed account of the pertinent observations.

MATERIAL AND METHODS

This report is based largely on observations made in six young adult *Macaca mulatta* monkeys in which surgical lesions had been placed in the amygdaloid complex. Several further incidental observations made in other material will be mentioned in the Discussion.

The operative procedure was as follows. With the animal in deep pentobarbital anaesthesia, unilateral lesions of the amygdaloid complex were made, using the subfrontal approach indicated by Scoville & Milner (1957). According to this procedure, a large frontal bone flap was turned, the dura opened widely, and the frontal lobe lifted from the orbital roof, with all exposed cortex thoroughly protected from

mechanical injury by strips of cottonoid soaked in saline. Gentle elevation of the fronto-temporal junction exposed the rostral aspect of the amygdalo-piriform prominence, and a narrow suction tip, fashioned of a suitably curved 20-gauge injection cannula with the cutting tip ground off, was inserted into the amygdalo-piriform complex, using the internal carotid and middle cerebral arteries respectively as medial and dorsal landmarks. Following the aspiration of amygdaloid substance through the small puncture hole, the dura was carefully sutured and the bone flap replaced and fastened.

The animals were killed by an overdose of pentobarbital 9–12 days post-operatively. Fixation of the brain with formalin was initiated by perfusion and extended by storage in the fixative for 6–12 weeks. Frozen sections of the brains were stained following the Laidlaw modification of the Nauta–Gygax silver technique, and axon degeneration, as identified microscopically, was recorded in projection drawings of selected sections.

OBSERVATIONS

As expected, marked variations were encountered in the localization of the lesions. In all cases, the suction tip had penetrated either the piriform cortex or the cortical amygdaloid nucleus, and the lesion extended laterally from this point of entry into at least the basal and accessory basal nuclei. The lateral nucleus was involved in four animals, the medial nucleus in one. In two cases the defect was found to encroach upon the temporal white matter covering the lateral aspect of the amygdala; one of these cases showed additional slight involvement of the ventral edge of the putamen. In all remaining cases the lesion was entirely confined to the amygdalo-piriform complex.

Further variations encountered in this series of experiments concerned the position of the lesion with respect to the dorso-ventral and rostro-caudal coordinates. As concerns the ventral amygdalofugal pathways, such variations appeared to affect the quantity rather than the distribution of the fibre degeneration, more massive degeneration apparently being related to greater involvement of the dorsal amygdaloid regions. The degeneration observed in the stria terminalis appeared to be more strikingly dependent on the localization of the lesion in regard to both quantity and distribution.

In the following account two of the six cases will be discussed in some detail.

(1) *Case MA* 3 (Text-fig. 1)

The lesion in this case was largely limited to the rostro-ventral quarter of the amygdaloid complex. It involved mainly the basal and accessory basal nuclei, and to a lesser extent the lateral nucleus. The suction tip had caused additional damage to the cortical nucleus and, more rostrally, to the piriform cortex. The lesion was separated from the temporal white matter adjoining the amygdaloid complex laterally by at least a millimeter of apparently normal tissue.

A. *Ventral amygdalofugal pathways*

It is evident even under low magnification that massive fibre degeneration extends from the lesion in the dorsal and rostral directions. Within the amygdaloid complex such degenerated fibres compose a complicated mazework (Text-fig. 1*b*) in which,

Text-fig. 1. Fibre degeneration observed in case MA 3. The amygdaloid lesion is indicated in jet black. Coarse dots indicate degenerating fibres of passage, fine stipple preterminal and terminal degeneration. Abbreviations: see p. 531.

however, at least one more condensed group of relatively fine fibres can be distinguished immediately dorsal to the basal amygdaloid nuclei (Text-fig. 1*a*). This fibre group is tentatively identified as the longitudinal association bundle, but it is noted that the bundle forms part of a more diffuse and widespread fibre system apparently originating mainly from the basolateral group of nuclei. It is of further interest to note the presence of numerous scattered degenerating axons of heavy calibre which, like the finer constituents of the longitudinal association bundle, in general follow dorsal and rostral trajectories through the amygdala.

Substantia innominata. From the level of the caudal border of the chiasma rostralward, large numbers of degenerating fibres curve medially and enter the sublenticular region often labelled substantia innominata. The latter region is pervaded by innumerable disintegrating axons of various calibres (Text-fig. 1*c*; Pl. 1*a*). In the ventral parts of the region fine fibres predominate, whereas coarse axons are more numerous in the dorsal zone adjoining the lentiform nucleus. The occurrence of fine degenerating pericellular fibres indicates that some amygdalofugal fibres actually terminate in the substantia innominata, both in the large-celled dorsal area known as the nucleus ansae peduncularis (Meynert's 'Basalganglion'; Ganser's nucleus basalis) and in the less well defined ventral zones which include a caudal extension of the nucleus of Broca's diagonal band. However, most of the fibres of the ventral amygdalofugal pathway only pass through the substantia innominata *en route* to more distant structures. Somewhat schematically, it can be said that such transit fibres are disposed in fan-tail fashion, with the caudal components oriented medially, the rostral ones rostrally in nearly sagittal planes.

Preoptic region and hypothalamus. The more caudal sublenticular transit fibres are distributed to the lateral preoptic and hypothalamic regions adjoining the substantia innominata (Text-fig. 1*a–c*). They terminate diffusely among cell groups scattered between the longitudinal fibres of the medial forebrain bundle. Only few such fibres accompany the medial forebrain bundle caudalward, and consequently little, if any, degeneration appears in the lateral hypothalamus at infundibular levels or farther caudally.

The most ventral of these amygdalo-hypothalamic fibres course immediately dorsal to the supraoptic nucleus, and a few scattered degenerating elements are seen to enter this cell group. There is, however, no convincing evidence for arborization of such fibres within the nucleus. Signs of fibre termination are, by contrast, abundant in the ventral hypothalamic zone immediately dorsal to the supraoptic nucleus.

Thalamus. The fibre degeneration extending to the lateral preoptic region is accompanied dorsally by a considerable number of degenerating coarse axons which join the inferior thalamic peduncle (Text-fig. 1*c*). Before gaining the peduncle most of these fibres follow dorsal paths in their medial course through the substantia innominata; some of the most dorsal ones even trace weaving trajectories through a ventral zone of the globus pallidus without, however, displaying signs of termination in that structure. In the peduncle the degenerating fibres form a rather scattered group (Pl. 1*b*) which, upon entering the thalamus, issues a few fibres medially to the nucleus reuniens (Text-fig. 1*c*) and laterally to the medial part of the nucleus reticularis thalami. The bundle then breaks up into a number of scattered fascicles

which curve caudalward and follow the medial one-third of the internal medullary lamina to the medial, magnocellular part of the ipsilateral dorso-medial thalamic nucleus (Text-fig. 1*a*, *b*). In this cell group the constituent coarse axons terminate with profuse pericellular arborizations, as indicated by the extremely dense feltwork of disintegrating fine axons which fills the nucleus and sharply delimits it from its surroundings (Pl. 1*c*). A small number of amygdalo-thalamic fibres decussate in the internal medullary lamina and disperse in the medial part of the contralateral dorso-medial nucleus.

Olfactory tubercle, septal region, gyrus subcallosus, rostral limbic cortex. Rostrally the substantia innominata continues into the region of the substantia perforata anterior. This region is characterized by the appearance of the olfactory tubercle, a circumscript cortical formation flanked medially by the nucleus of Broca's diagonal band, and laterally by the prepiriform cortex (Text-fig. 1*d*). In its caudal half the tubercle is separated from the more dorsally situated lentiform nucleus by a rather loosely structured rostral extension of the nucleus ansae peduncularis (n. basalis of Ganser). More rostrally, however, the diagonal band curves dorsally into the septal region, and the nucleus ansae peduncularis tapers to a vague rostral limit, leaving the olfactory tubercle in immediate contact with the ventral aspect of the caudato-putaminal junction. Farther rostrally still the olfactory tubercle reaches its rostral boundary; from here forward the fundus striati is covered by the orbito-frontal cortex.

As shown by Text-fig. 1*d*, numerous fibres of the sublenticular amygdalofugal fibre system extend forward into the region of the olfactory tubercle. In the tubercle proper such fibres appear to terminate in the multiform as well as in the pyramidal cell layers. Other fibres are distributed to the nucleus ansae peduncularis deep to the tubercle, whereas densely packed fine degenerating axons follow a more medial path alongside and in the diagonal band. Undoubtedly many of these more medial fibres terminate in the diagonal nucleus, but this degeneration does not follow the nucleus over more than a short distance into the septal region (Text-fig. 1*e*), and consequently no axon degeneration is detectable in more dorsal parts of the septum.

Fibres of the same medial group that conveys amygdaloid efferents to the nucleus of the diagonal band extend forward beyond the septum, in the white matter of the gyrus rectus (Text-fig. 1*e*). Many of these fibres arborize in the grey matter of the gyrus subcallosus (Text-fig. 1*f*). Other fibres of the same group continue even farther forward, bend around the genu corporis callosi and become dispersed among the fibres of the fasciculus cinguli. Degenerating arborizations of these long amygdalofugal fibres are found scattered in the ventral region of approximately the rostral one-third of the gyrus cinguli.

Temporal cortex, insula, putamen, claustrum, orbito-frontal cortex. As shown by Text-fig. 1*e*–*f*, the aforementioned degeneration spreading to the septum and rostral regions of the gyrus fornicatus forms only a medial part of a widespread stratum of degenerating fibres that covers the ventral half of the putamen and nucleus accumbens. Approximately the lateral half of this degenerated fibre stratum appears to be made up of fibres closely related to the fasciculus uncinatus and the ventral margins of the outer capsules. It seems likely that these degenerating axons belong to a fibre system which takes a lateral exit from the amygdaloid complex, enters the white

matter of the temporal lobe (Text-fig. 1*b*) and spreads from here in various directions: (*a*) ventralward to a rostral part of the inferior temporal gyrus, (*b*) lateralward to rostral parts of the middle and superior temporal gyri, and (*c*) rostrally to the claustrum (Text-fig. 1*b*–*e*), the ventral part of the insular cortex (Text-fig. 1*b*, *d*, *e*), the lateral zone of the putamen (Text-fig. 1*e*–*f*), and the caudal orbito-frontal cortex (Text-fig. 1*f*). It must be remarked, however, that with the exception of the fibres to the inferior temporal gyrus and claustrum, a continuous tracing of the paths followed by the degeneration in question has not been possible. It is especially difficult to establish the connexion of the degeneration in the temporal white matter with that in the fasciculus uncinatus and in the base of the outer capsules. Hence, as will be pointed out more fully in the Discussion, the evidence in regard to some of the amygdalo-cortical connexions indicated above appears to be somewhat less than conclusive.

B. *Stria terminalis*

In this case only a small ventro-lateral part of the stria terminalis is degenerated. The fibres involved extend forward to the bed nucleus of the stria, in which cell groups all appear to terminate (Text-fig. 1*d*). No strial degeneration can be traced ventralward past the anterior commissure.

(2) *Case MA* 11 (Text-figs. 2, 3)

This case is briefly described here for the supplementary information which it furnishes concerning the stria terminalis. The lesion in MA11 was situated farther caudally in the amygdaloid complex than was the case in MA3, and it extended into that part of the complex which extends caudally in the roof of the temporal horn of the lateral ventricle (Text-fig. 2). As in MA3, the lesion involved mostly the basolateral cell groups, but it extended farther dorsally; also it had spared the rostral half of the amygdaloid complex, which was involved to a considerable extent in MA3.

Stria terminalis

The stria terminalis shows massive degeneration. In contrast to its localization in case MA3, the degeneration is densest in the dorso-medial half of the stria; it maintains this relative position throughout its course through the bed nucleus of the stria (Text-fig. 3*a*). Few if any of the disintegrating fibres appear to end in the latter nucleus, and virtually all continue around the rostral and caudal aspects of the anterior commissure into the medial preoptic region and beyond it into the hypothalamus (Text-fig. 3*b*, *c*). Most of these hypothalamic stria fibres appear to distribute to the anterior hypothalamic nucleus (Text-fig. 3*b*; Pl. 1*b*). In their course caudalward through this nucleus the degenerating fibres shift progressively farther ventrally, rapidly decreasing in number. In frontal sections involving the caudal one-third of the optic chiasma only a small number have remained. These strial components are here found scattered in regions ventral and somewhat lateral to the rostral pole of the ventro-medial nucleus (Text-fig. 3*c*). Only a few isolated degenerating fibres can be traced some distance beyond the caudal border of the

chiasma; their distribution area appears to be ventral to the ventro-medial nucleus and possibly involves a small lateral part of the arcuate nucleus. No degenerating fibres can be traced into the ventro-medial nucleus proper.

The only further component of the stria terminalis which is recognizable in this case appears in the form of a small number of degenerating fibres which join the dorsal stratum of the anterior commissure (Text-fig. 3*a*). Although these few fibres can be followed across the midline, it is not possible to establish their ultimate

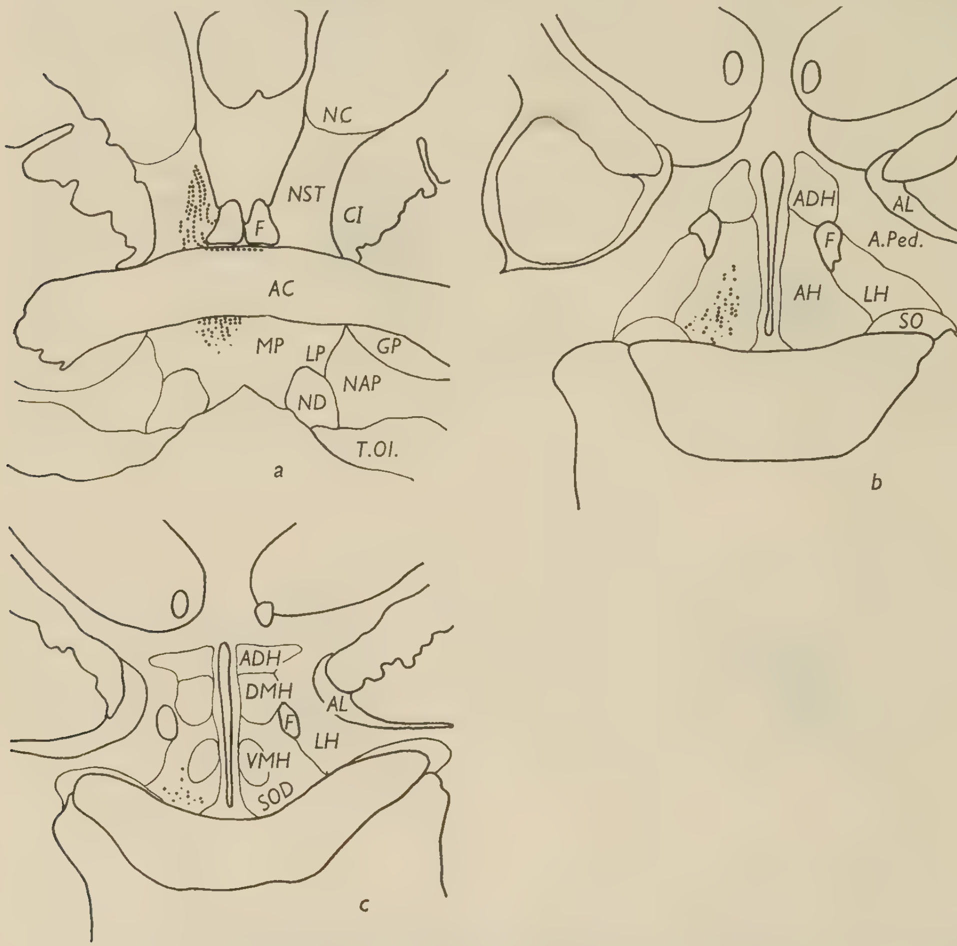
Text-fig. 2. Four drawings of transverse sections showing the extent of the amygdaloid lesions in case MA 11. Complete tissue loss is indicated in jet black, heavy gliosis with loss of cell bodies by cross-hatching. Abbreviations: see p. 531.

distribution. It is likely that this scant degeneration corresponds to the much more massive pars commissuralis striae terminalis described in the opossum by Johnston (1923), and in several subprimate mammalian forms by others.

Ventral projection pathways

The degenerating ventral amygdalofugal system charted in this case appears similar to that observed in MA 3, with the exception that the degeneration in several of the rostral components of the system is considerably less massive than in MA 3.

This is especially true of the degenerations traceable to the orbito-frontal cortex, putamen, claustrum, and olfactory tubercle. By contrast, the degeneration distributing to the nucleus of Broca's diagonal band, as well as to the gyrus subcallosus and rostral limbic cortex appears comparable in volume to that observed in MA3.



Text-fig. 3. Degeneration of the stria terminalis as observed in case MA 11. Symbols as in Text-fig. 1. Abbreviations: see p. 531.

DISCUSSION

The amygdaloid complex, as is well known, consists of several cell groups of various architecture, and it is unlikely that all these nuclei contribute in the same manner to the pathways described in the foregoing account. Unfortunately, even small surgical lesions in heterogeneous structures such as the amygdala destroy not only the cell bodies which fall within their boundaries, but also fibres of passage originating outside the area of the lesion proper. Only gross impressions of the mosaic of origin can therefore be expected from studies involving the surgical production of amygdaloid lesions. Apart from this restriction, the particular surgical procedure followed

in the present study has the advantage of obviating the track-damage to other structures which unavoidably complicates stereotactic lesions and which would have vitiated several of the present observations by involving either the internal capsule or the temporal white matter lateral to the amygdala.

There remains, none the less, reason for caution in the interpretation of fibre degenerations following amygdaloid lesions: such lesions almost certainly involve transit projection fibres from the piriform cortex. The production of selective defects of the monkey's periamygdaloid cortex, which could have helped to clarify this point, has so far been unsuccessful in our hands. No attempt can consequently be made at this time to distinguish between paleocortical fibres passing through the amygdala, and projection fibres from the amygdaloid cell groups proper. Hence, although the term 'amygdalofugal pathways' is used here, the connexions in question are perhaps more cautiously interpreted as projections from the amygdalo-piriform complex as a whole.

The observations reported above have confirmed the existence of two main amygdalo-subcortical projection pathways in the macaque, namely the relatively compact stria terminalis, and a much more diffuse and widespread ventral amygdalofugal fibre system. Furthermore, the present findings suggest the existence of a third efferent pathway which connects the amygdala with several cortical regions.

As the literature pertaining to the fibre connexions of the amygdala has recently been reviewed succinctly by Gloor (1955, 1959), a detailed bibliographical survey would seem superfluous at this time. In the following account references will therefore be limited mostly to those previous experimental observations which seem immediately pertinent to the findings described in the foregoing account.

A. *Ventral amygdalofugal pathways*

The present findings emphasize the great volume of the ventral amygdalo-subcortical pathways, as well as the multiplicity of their connexions. As pointed out in the description of case MA3, this fibre system, corresponding in part at least to Johnston's (1923) longitudinal association bundle, emerges from the amygdala in the dorso-medial direction. It spreads medially and forward underneath the lentiform nucleus, distributing fibres to (*a*) basal forebrain structures, namely, the substantia innominata, the lateral preoptic and hypothalamic areas, the substantia perforata anterior including the olfactory tubercle, and the nucleus of the diagonal band; (*b*) rostral parts of the gyrus fornicatus: subcallosal gyrus and anterior cingulate cortex; and (*c*) the magnocellular element of the dorso-medial thalamic nucleus, via the ansa peduncularis.

Amygdaloid projections to the septal-lateral preoptic-lateral hypothalamic region can presumably serve to lead impulses of amygdaloid origin into the paths of both the medial forebrain bundle and stria medullaris-fasciculus retroflexus. The same would appear to hold for the amygdaloid pathways to the substantia innominata, for there is evidence that this structure also contributes a large number of fibres to the medial forebrain bundle (Mehler & Nauta, unpublished observations in the monkey). By virtue of these synaptic relationships with several sources of origin of the medial forebrain bundle and related fibre trajectories such as the stria medullaris, the ventral amygdalo-subcortical pathway appears comparable to the fornix system. Much

like the latter, it appears to furnish a first link in a multi-synaptic chain of conduction which, partly through the intermediary of the lateral hypothalamus, reciprocally connects the limbic forebrain region with an extensive medial area of the midbrain tegmentum and central grey midbrain substance. As indicated previously (Nauta, 1958), this 'limbic system–midbrain circuit' is connected by massive escape pathways with the more laterally located central mesencephalic and subthalamic reticular formation, connexions by which the limbic forebrain structures could conceivably affect a diversity of reticular mechanisms in addition to the autonomic and endocrine functions represented in the midbrain and hypothalamus. It seems possible, for example, that such complex motor stereotypes as the licking, sniffing and chewing movements which have been observed in the course of amygdaloid stimulation experiments (see Gloor, 1959) were elicited by the medium of such indirect amygdalo-reticular connexions.

It is interesting to compare the foregoing anatomical considerations with the results of Gloor's (1955) electrophysiological study in the cat. The distribution of responses of shortest latency (less than 7 msec.) recorded by Gloor in the septum, preoptic region and anterior hypothalamus agrees well with the spread of the ventral amygdalo-subcortical system observed in the present study. Responses of longer latency, presumably denoting indirect connexions, were recorded from more caudal hypothalamic areas, as well as from a large expanse of mesencephalic reticular formation. Although Gloor's observations in general corroborate the anatomical evidence of widespread, if indirect, amygdalo-reticular pathways, it is only fair to point out that his results do not support the notion that such connexions are established mainly by the medial forebrain bundle or via the stria medullaris-fasciculus retroflexus trajectory. Widely scattered responses to amygdaloid stimulation were, for example, recorded from the mesencephalic tegmentum with delays considerably shorter than those registered in caudal regions of the lateral hypothalamus, i.e. along the more caudal part of the hypothalamic trajectory of the medial forebrain bundle. Actually, Gloor's electrophysiological evidence appears to point to extremely medially placed pathways, spreading caudalward through the periventricular hypothalamic region, as the more direct amygdalo-mesencephalic conductors. Conceivably, one such pathway could be furnished by amygdalo-hypothalamic fibres articulating with descending components of Schütz's periventricular fibre system. However, even the relatively fast potentials recorded along this medial route fail to match the surprisingly short latency of some widespread responses obtained in Gloor's experiments from the rostral midbrain tegmentum. In commenting on these findings, Gloor himself suggests the possible existence of a direct or at most oligosynaptic amygdalo-tegmental pathway by-passing the hypothalamus. If such a connexion indeed exists, it appears likely that it would follow the internal capsule and cerebral peduncle. At this time, however, one is forced to conclude that no data are available which could explain all the details of Gloor's observations in terms of known anatomical pathways.

Amygdalo-hippocampal connexions. The present study has failed to produce evidence of the direct amygdalo-hippocampal connexions which have been described on the basis of observations in normal material (Hilpert, 1928, and others). However, the existence of alternate pathways subserving amygdalo-hippocampal interaction

is made likely by the conspicuous amygdaloid projection to the nucleus of the diagonal band, a cell group which is believed to project to the hippocampus either directly (Daitz & Powell, 1954) or via the presubiculum (Cragg & Hamlyn, 1957). The pathway in question probably conducts in both directions: the hippocampal formation is known to project to the entire septal region, and from the latter fibres can be followed along the diagonal band to the immediate vicinity of the amygdala, in the monkey even directly into the medial amygdaloid region (Valenstein & Nauta, 1959). Amygdalo-hippocampal conduction via the entorhinal area as suggested by Gloor (1955) seems conceivable also, although the evidence regarding this pathway is controversial. Adey & Meyer (1952) failed to obtain anatomical evidence of connexions from the amygdalo-piriform complex to the entorhinal area. On the other hand, the spread of strychnine spikes from the periamygdaloid cortex to caudal regions of the hippocampal gyrus, observed by Pribram & MacLean (1952), tends to support Gloor's suggestion.

Amygdalo-thalamic connexions. In agreement with Fox's (1949) observations in Marchi experiments in the monkey, a quite massive amygdaloid projection could be traced via the inferior thalamic peduncle to the dorso-medial nucleus of the thalamus. As degeneration was found in this pathway in all of the six cases of amygdaloid lesion, it is not possible to identify the contributing amygdaloid cell groups with certainty. It is, however, of interest that the lesions produced in the present study all involved the cortical nucleus, the accessory basal nucleus, and the basal nucleus proper. The heavy calibre of most of the constituent axons tends to suggest the basal and lateral nuclei as the most likely sources of origin of the amygdalo-thalamic connexion.

Although not specifically mentioned by Fox, it is clear from the present findings that the amygdalo-thalamic pathway terminates almost exclusively in the medial, magnocellular division of the dorso-medial nucleus. Some further sparse termination seems to take place in the rostral midline region and in the paracentral intralaminar nucleus. No fibres of the connexion could, however, be identified in the lateral part of the dorso-medial nucleus.

The detailed analysis of the dorso-medial thalamo-cortical projection by Pribram, Chow & Semmes (1948) has shown that the medial, magnocellular component of the dorso-medial nucleus projects specifically upon the orbito-frontal cortex. The present findings thus suggest that Fox's amygdalo-thalamic tract represents the first link in a major transthalamic amygdalo-orbito-frontal connexion. The extremely dense arborization of the amygdaloid projection fibres in the nucleus suggests furthermore that fibres of amygdaloid origin furnish the major afferent supply to the medial element of the dorso-medial thalamic nucleus. It must, however, be noted that other, apparently less massive pathways to the pars medialis of the dorso-medial nucleus, have been traced from the septal region (Guillery (1959) in the cat; Valenstein & Nauta (1959) in the monkey), and from the inferior temporal gyrus (Whitlock & Nauta (1956) in the monkey). The temporal cortical projection to the dorso-medial nucleus, like that from the amygdala, follows the inferior thalamic peduncle, but it is unlikely that it was involved in the present experiments as it courses lateral to the amygdala in the white matter of the temporal lobe.

It is noteworthy that in the cat no amygdalo-thalamic projections have been

identified by either experimental-anatomical (Fox, 1943; Lammers & Lohman, 1957; Hall, 1960) or electrophysiological (Gloor, 1955) methods. However, thalamopetal fibre degeneration entirely comparable to the present findings in the monkey has been produced in the cat by lesions in the preoptic region (Nauta, 1958) and substantia innominata, structures that both receive numerous fibres from the amygdala. It thus appears that, despite the apparent absence of a direct connexion, an anatomical pathway for amygdalo-thalamic conduction is present in the cat also. Comparable interspecific variations in neuronal organization have been noted in various other connexions related to the limbic system (Valenstein & Nauta, 1959). It is tempting to speculate that such anatomical differences between species could reflect important functional variations in the neural mechanisms concerned.

Amygdaloid projections to the pulvinar as mentioned by Fox (1949) could not be identified in the present study. Projections apparently comparable to that observed by Fox have, however, been traced from a large extent of the temporal cortex (Whitlock & Nauta, 1956). As Fox's observations were reported only in the form of an abstract it is not possible to say to what extent degeneration of fibres to the pulvinar could have been caused in his experiments by surgical involvement of such cortico-thalamic connexions.

Amygdalo-cortical connexions. In several of the cases of amygdaloid lesion here studied axon degeneration could be traced in continuity from the lesion to (*a*) the gyrus subcallosus and rostral cingulate cortex, and (*b*) the rostral half of the inferior temporal gyrus. The former connexion, established by a moderate number of axons which accompany the fibre pathway to the nucleus of the diagonal band, would seem to correspond to the amygdaloid projection to the gyrus subcallosus observed in the cat by Lammers & Lohman (1957). As regards the fibres to the inferior temporal gyrus, there is little reason to suspect that their degeneration could have been caused by non-specific factors, for besides the uncus no part of the temporal lobe was actually touched during surgery. It would thus seem justified to accept the existence of a rather sparse and diffuse projection of the amygdaloid complex to the inferior temporal gyrus, a connexion which reciprocates an apparently somewhat more massive cortico-amygdaloid projection arising in the same general region of the temporal cortex (Whitlock & Nauta, 1956).

Less unequivocal are the present data regarding amygdaloid pathways to rostral parts of the middle and superior temporal gyri, to the ventral insular region, and to the caudal orbito-frontal cortex. In all of the present cases fibre degeneration was observed to spread to these cortical regions, apparently largely via the uncinate fasciculus, but it was impossible to trace it in continuity from the amygdaloid lesion. Naturally, this failure could have resulted from a peculiar (e.g. recurrent and diffuse) mode of junction of amygdalofugal fibres with components of the uncinate bundle. On the other hand, however, the possibility must be considered that the degeneration in question was caused by inadvertent damage to the orbito-frontal cortex inflicted during the surgical procedure. Such damage could have caused the degeneration of temporopetal fibres in the uncinate fasciculus, and could at the same time have mirrored the amygdaloid projection to the orbito-frontal cortex suggested by the present findings. The circumstance that microscopic evidence of punctate lesions in the orbito-frontal cortex could be found in only one of the cases does not

entirely preclude this possibility.* On the other hand again, the suspicion of orbito-frontal lesion is contradicted on one important point: whereas surgically produced lesions of the orbito-frontal cortex are followed by conspicuous fibre degeneration in the internal capsule, capsular degeneration was absent in all but one of the present cases. It is difficult to conceive of orbito-frontal lesions which could have caused substantial degeneration of associated cortical efferents without concomitant disintegration of subcortical projection fibres. Furthermore, in the present experiments the amount of degeneration in the orbito-frontal cortex appeared to be dependent on the extent of damage inflicted to the rostral half of the amygdaloid complex. It was, for example, notably larger in case MA 3 than in MA 11 in which the lesion was confined to the caudal half of the complex. The surgical procedure being the same in all cases, this observation suggests the existence of a true amygdaloid projection to the orbito-frontal cortex, arising largely in the rostral half of the amygdaloid complex.

In conclusion, the weight of evidence appears to favour the actual existence of amygdaloid projections to (*a*) rostral parts of the middle and superior temporal gyri, (*b*) a ventral region of the insular cortex, and (*c*) a large extent of the caudal orbito-frontal cortex. Some reserve in accepting the present evidence of these amygdalo-cortical connexions remains necessary, especially because it has not been possible to trace the pathways in question in continuity. However, even if the presence of the direct amygdalo-cortical connexions in question be discounted there can be little doubt that the amygdaloid complex can influence the neural mechanisms of at least the orbito-frontal cortex through the intermediary of the dorso-medial thalamic nucleus.†

B. *Stria terminalis*

For detailed normal anatomical descriptions of the various components of the stria terminalis in several subprimate forms especial reference is made to the publications of Johnston (1923), Berkelbach v. d. Sprenkel (1926), Humphrey (1936), and Ariens Kappers, Huber & Crosby (1936).

From a comparison of cases MA 3 and MA 11 it is apparent that the stria terminalis originates largely in the caudal half of the amygdaloid complex, a finding which agrees well with Fox's (1943) and Adey & Meyer's (1952) conclusions from previous experimental studies. The present observations suggest further that some stria fibres, originating in the rostral half of the complex, do not extend beyond the bed nucleus of the stria and hence do not contribute to the preoptic and hypothalamic components of the system. Conversely, the findings in MA 11 indicate that stria

* This statement is based on our experience that strong elevation of the occipitotemporal cortex in the cat, even if carried out extradurally, can cause massive intracortical and corticofugal fibre degeneration despite the absence of identifiable gross or histological lesions of the cortex.

† After this discussion was written, Drs J. Klingler and P. Gloor kindly sent us the typescript of a gross-anatomical study of temporal lobe connexions in man, performed by the aid of Klingler's dissection technique. In this study fibre tracts were dissected which extend between the amygdala on the one hand, the tip of the temporal lobe, the insula and the orbito-frontal cortex on the other hand. The pathways in question emerge from the lateral side of the amygdala and follow curved trajectories in close relationship to the uncinate fasciculus. Drs Klingler and Gloor point out that the gross dissection technique can offer little information regarding the polarity of fibre connexions. However, the appearance of the fibre tracts demonstrated by their analysis is in several respects consistent with the experimental evidence of amygdalo-cortical pathways discussed above.

fibres of more caudal origin by-pass the bed nucleus and make up the bulk of the preoptic and hypothalamic components.

This study has failed to confirm the existence of supracommissural fibres of the stria terminalis to the septal region. The possibility cannot be excluded that such fibres originate in amygdaloid regions not involved in the present experiments. In a case of complete surgical interruption of the stria several millimetres caudal to the anterior commissure (case MF13), fibre degeneration was found in the septal region, but this finding was considered inconclusive for the reason that the fimbria fornicis was to some extent involved in the lesion.

The commissural component of the stria terminalis would seem to be of minimal volume in the monkey. Both in case MA11 and in the case of stria terminalis section (MF13) mentioned above, only a few degenerating fibres could be followed across the midline in the dorsal stratum of the anterior commissure. As in previous Marchi studies in the cat (Fox, 1943; Ban & Omukai, 1959), the termination of these commissural fibres could not be determined.

Stria terminalis fibres to the preoptic region and hypothalamus appear to form by far the largest component of the stria in the monkey. According to the present findings, such fibres distribute largely, if not exclusively, to the medial zone of the preoptic and hypothalamic regions. The stria terminalis differs in this respect from the ventral amygdalo-hypothalamic pathway which appears to connect primarily with more lateral preoptico-hypothalamic areas, and specifically with the region interstitial to the medial forebrain bundle. Within the medial zone most of the stria terminalis fibres appear to terminate among the cells of the medial preoptic and anterior hypothalamic nuclei. Only few fibres could be followed farther caudalward, and all of these appeared to terminate at and only slightly behind the caudal border of the optic chiasma, in the extreme ventral hypothalamic region containing the scattered cells of the so-called nucleus supraopticus diffusus (Rioch, Wislocki & O'Leary, 1940). Some of the longest stria fibres may end in contact with the arcuate nucleus of the infundibulum.

From an experimental study by the Glees technique in the monkey, Adey & Meyer (1952) concluded that amygdalo-hypothalamic fibres are distributed in large part to the ventro-medial hypothalamic nucleus. The same study furthermore indicated a virtually symmetrical bilateral distribution of the amygdalo-hypothalamic projection. The present observations differ from these conclusions in major respects. Naturally, in comparing the present findings with those of Adey & Meyer the possibility must be considered that certain amygdalofugal fibre contingents had escaped degeneration in all of the present cases of amygdaloid lesion. However, incidental findings made in three further cases likewise failed to confirm the existence of bilateral amygdaloid projections to the ventro-medial hypothalamic nucleus. In one of these supplementary cases (MF13 mentioned before in this Discussion) complete unilateral interruption of the stria terminalis resulted in exclusively ipsilateral degeneration of hypothalamic stria fibres in a distribution comparable to that found in MA11. Two other cases, in which the lateral half of the substantia innominata had been extensively damaged in an unsuccessful attempt to produce stereotactic lesions of the globus pallidus, again showed absence of any but ipsilateral hypothalamic degeneration; in neither case was fibre degeneration observed in the

ventro-medial nucleus. The lesions in the two latter cases had undoubtedly severed most of the ventral amygdalo-hypothalamic fibres in their sublenticular passage medialward. Thus, in the present experiments neither a variety of amygdaloid lesions nor the massive interruption of the two known amygdalo-hypothalamic pathways was followed by contralateral hypothalamic fibre degeneration or degeneration of preterminal fibres in the ventro-medial nucleus of either side. Even when allowance is made for the greater ease with which details of terminal degeneration can be identified with the Glees method than by the Nauta–Gygax technique (Bowsher, Brodal & Walberg, 1960), the very small number of the degenerated fibres of passage which in the present study could be followed into the medial hypothalamic zone caudal to the optic chiasma appears to contradict the existence of a significant direct amygdaloid projection to the ventro-medial hypothalamic nucleus.

SUMMARY

The fibre degenerations resulting from lesions in the amygdaloid complex in the monkey were studied by means of the Nauta–Gygax technique. The results confirm the existence of two major amygdalo-subcortical fibre systems, namely, a relatively diffuse ventral amygdalofugal pathway, and the compact stria terminalis.

1. The ventral amygdalofugal pathway, apparently the most massive amygdaloid projection system, spreads medially and forward ventral to the lentiform nucleus and connects with the substantia innominata, lateral preoptic and hypothalamic regions, nucleus of Broca's diagonal band, and the olfactory tubercle. A prominent further component of the system by-passes the preoptic region and follows the inferior thalamic peduncle to terminate in the medial, magnocellular division of the dorso-medial thalamic nucleus. Furthermore, the ventral amygdalofugal pathway contains an amygdalo-cortical component which accompanies the pathway to the nucleus of Broca's diagonal band and terminates in rostral parts of the gyrus fornicatus (gyrus subcallosus and rostral cingulate cortex).

2. The stria terminalis originates mostly in the caudal half of the amygdaloid complex. Fibres arising most rostrally in the complex appear to terminate largely in the bed nucleus of the stria terminalis. Other fibres of more caudal origin form a prominent preoptico-hypothalamic component distributing fibres to the medial preoptic nucleus, anterior hypothalamic nucleus, and the region of the nucleus supraopticus diffusus. No stria terminalis fibres could be followed to the ventro-medial hypothalamic nucleus. Only ipsilateral amygdalo-hypothalamic fibres could be identified.

3. Evidence was obtained of an additional amygdalofugal fibre system which emerges through the lateral and ventral sides of the amygdala and distributes fibres to rostral parts of the superior, middle and inferior temporal gyri, ventral insular cortex, claustrum, rostral putamen, and caudal orbito-frontal cortex. As most of these connexions could not be followed in continuity, the present evidence of their existence cannot by itself be considered conclusive.

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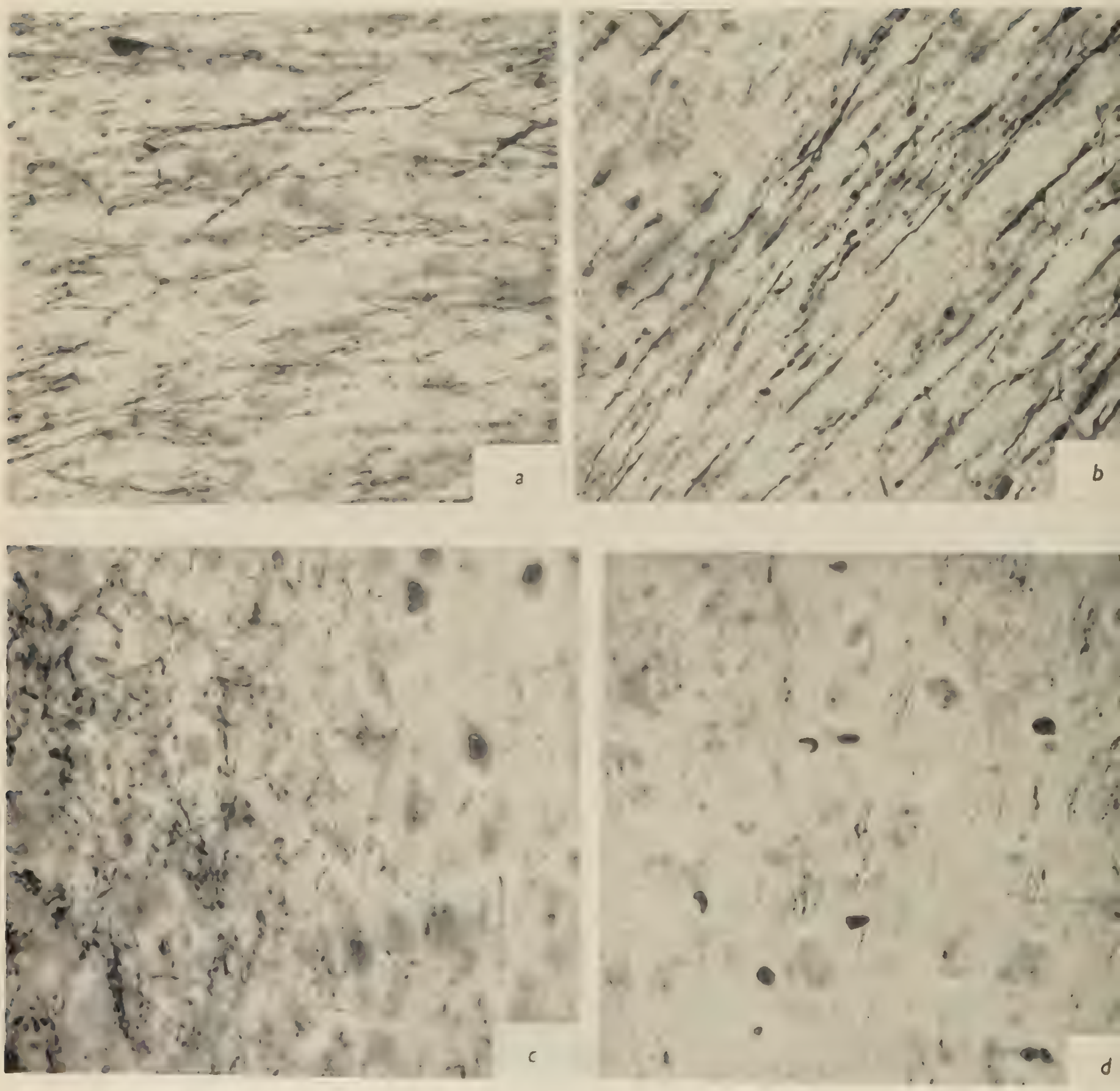

NAUTA—Amygdaloid projections in the monkey

(*Facing p.* 531)

EXPLANATION OF PLATE

Photomicrographs (× 230) of fibre degeneration following lesion of the amygdaloid complex.

a, degenerating axons of various calibre traversing the substantia innominata. Case MA3.
b, degenerating coarse axons in the inferior thalamic peduncle. Case MA3.
c, dense pericellular and intercellular axon degeneration in a circumscript region (pars medialis) of the n. dorsomedialis thalami. The nucleus periventricularis anterior thalami appears near the right margin of the picture. Case MA3.
d, degenerating fascicles of the stria terminalis passing through the anterior hypothalamic nucleus. Case MA11.

KEY TO ABBREVIATIONS USED IN FIGURES

AC, anterior commissure; *ADH*, area dorsalis hypothalami; *AH*, nucleus anterior hypothalami; *AL*, ansa lenticularis; *AM*, nucleus anterior medialis thalami; *A.Ped.*, ansa peduncularis; *AV*, nucleus anterior ventralis thalami; *B*, basal amygdaloid nucleus; *CI*, capsula interna; *Cl*, claustrum; *DMH*, nucleus dorsomedialis hypothalami; *CO*, cortical amygdaloid nucleus; *DMm*, nucleus dorsomedialis thalami, pars medialis; *F*, fornix; *FU*, fasciculus uncinatus, *GP*, globus pallidus; *GR*, gyrus rectus; *Hp*, hippocampus; *L*, lateral amygdaloid nucleus; *LH*, nucleus lateralis hypothalami; *LP*, nucleus preopticus lateralis; *MP*, nucleus preopticus medialis; *NAP*, nucleus ansae peduncularis; *NC*, nucleus caudatus; *ND*, nucleus of the diagonal band of Broca; *NST*, nucleus striae terminalis; *NSt*, nucleus subthalamicus; *Pp*, cortex prepiriformis; *Put*, putamen; *SO*, nucleus supraopticus; *SOD*, nucleus supraopticus diffusus; *ST*, stria terminalis; *TO*, tractus opticus; *T.Ol.*, tuberculum olfactorium; *V*, lateral ventricle; *VA*, nucleus ventralis anterior thalami; *VL*, nucleus ventralis lateralis thalami; *VMH*, nucleus ventromedialis hypothalami.

CORTICO-STRIATE INTERRELATIONS IN THE ALBINO RAT

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INTRODUCTION

Interest in connexions between the cerebral cortex and corpus striatum has been revived by the work of Dusser de Barenne, Garol & McCulloch (1942) using the strychnine method. Although this work is now largely disregarded there has persisted the widespread concept that the cortical afferents to the striatum are derived from areas 4 and 6 (Jung & Hassler, 1960, pp. 869–70). In fact the contention of Dusser de Barenne and his colleagues that there is a differential projection to the caudate nucleus (from the strip areas) and the putamen (from areas 4 and 6) has never been critically investigated. Examination of the literature further emphasizes the confusion concerning the cortical areas involved and even the postulation that a connexion exists at all. Also the 'fibres of passage' explanation of degeneration seen in the striatum after cortical ablation, used by Wilson (1914) and others, has not been convincingly refuted.

Retrograde cell changes in the striatum after cortical ablation have rarely been found (Morrison, 1929). However, Minkowski (1923) reported obvious cell changes after cortical ablation and since that time there have appeared numerous physiological reports recording striato-cortical impulses (Hovde & Mettler, 1953; Purpura, Housepian & Grundfest, 1958).

In view of these discrepancies it has been decided to reinvestigate the problems of cortico-striate and striato-cortical connexions.

MATERIAL AND METHODS

Cortico-striate connexions

In all, thirty-six rats have been used and a variety of histological techniques employed.

The brains from ten animals of varying ages have been stained by a modified Golgi rapid method (Sholl, unpublished) and sectioned at 150μ. This technique is similar in essentials to method 1764 of Romeis (1948).

In a further nineteen rats localized cortical ablations have been made by suction or thermocoagulation. The position and extent of the lesions is recorded on diagrams similar to those of Lashley (1941), and Lashley's account of the motor areas and thalamo-cortical projections in the rat is used throughout. Three rats survived 10 days postoperatively, and the brains have been stained by Swank & Davenport's (1935) modification of the Marchi method, before sectioning at 15μ. One additional unoperated brain has served as control.

The other sixteen animals survived 5–7 days after operation, when the brains

* This work was carried out during the tenure of a University of London Postgraduate Studentship.

were perfused with 10 % formol saline and fixed for from 2 weeks to 6 months. These brains, together with six unoperated controls, have been sectioned either at 30 μ on a freezing microtome, or at 15 μ after embedding in paraffin. Frozen sections can be serialized by collecting individually in a tray divided into compartments; for staining sections are grouped *across* the series and are then readily reserialized. The sections were stained by the Nauta methods or a modification, as shown in Table 1. Brains used for other investigations, but stained by these techniques, have been available for comparison. The earlier method of Nauta & Gygax (1951) stains normal fibres, whereas the later method (1954) gives a differential impregnation of degenerating axons. It has been found, however, that the earlier technique stains, near their termination, degenerating fibres of smaller calibre than the finest demonstrated by the differential method, but impregnates poorly the main part of degenerating axons. The modification, for paraffin sections, by Guillery, Shirra & Webster (1961) gives results similar to the later Nauta method (1954), but has the advantage that sections can be serialized more readily and is, on the whole, less capricious in use.

Table 1. *Animals used to investigate cortico-striate connexions. Controls are not included*

Rat no.	Plane of section	Technique
1, 18, 22	Parasagittal	Nauta & Gygax (1954)
34, 46	Coronal	Guillery, Shirra & Webster (1961)
44	Parasagittal	
76, 87, 111	Coronal	Nauta & Gygax (1954)
86, 90, 91, 92	Parasagittal	
106, 112, 115	Coronal	Nauta & Gygax (1951, 1954)
68, 69, 70	Parasagittal	Swank & Davenport (Marchi) (1935)

Striato-cortical connexions

Cortical ablations have been made in five adult rats which survived for 10 weeks. The lesions are recorded as described above. After embedding in paraffin and sectioning at 15 μ, alternate one in ten sections have been stained with cresyl violet or with Holmes's (1943) or Peters's (1958) stain.

Eighteen brains from rats not older than 6 days at operation (Brodal, 1948) have been treated in a similar way, after the animals had survived from 3 to 12 days.

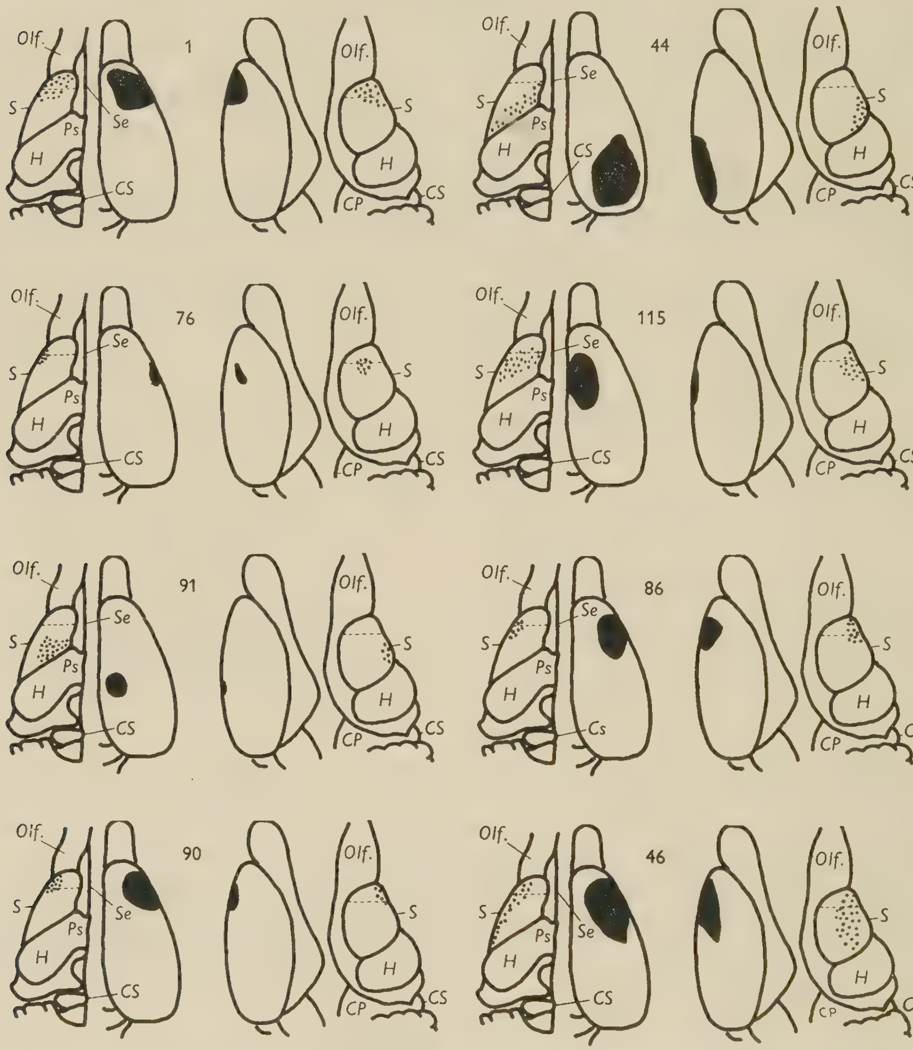
Several normal brains prepared as above, from adult and young animals, have been available for comparison.

RESULTS

Cortico-striate connexions

In young animals especially, the Golgi rapid method reveals collaterals leaving the internal capsule bundles and entering the striatum—which in the rat consists of a conjoined caudate nucleus and putamen with small bundles of internal capsule fibres passing through the single nucleus. Almost all these collateral branches are set roughly at right angles to the parent axons (Pl. 1, fig. 4) and are readily distinguished from fibres leaving and rejoining the internal capsule; occasional collaterals bifurcate (Pl. 1, fig. 5). These fibres may be ascending or descending, but it is not possible with the present material to discover the location of the parent cells.

In the material impregnated by Marchi's method the lesions are all large, frontal in position, and only one avoids damage to the underlying white matter. In one rat there is a small area of cell necrosis in the striatum. The internal capsule bundles

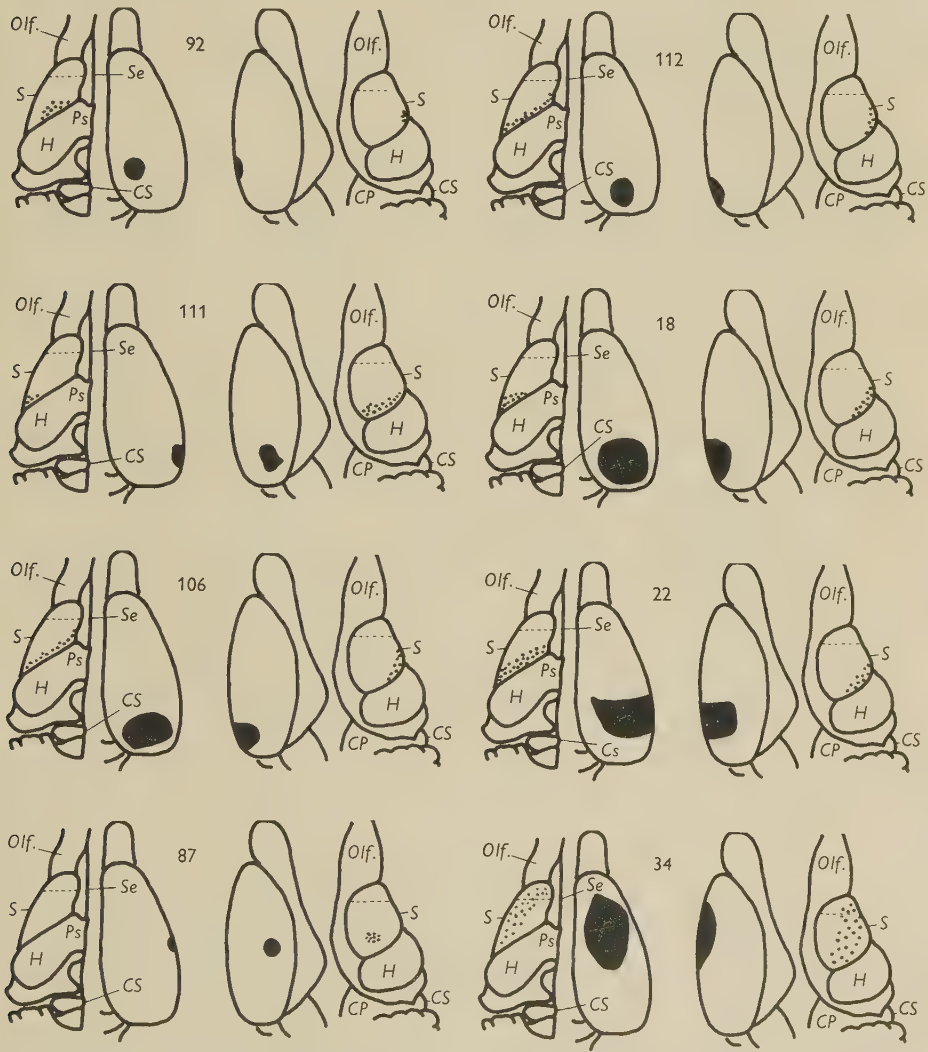


Text-fig. 1. Cortical lesions and resulting striatal degeneration as shown by silver methods. Each figure shows the cortical lesion viewed from above and from the side. On either side are diagrams of a decorticate rat brain viewed from above (on the left) and from the side (on the right), forming mirror images of the cortical diagrams. Degeneration is represented by dots in the striatum (*S*). The numbers are those used in the text. The projection is homolateral, the arrangement of diagrams being adapted for convenience.

of all three specimens contain osmium-stained droplets but the surrounding striatum is free of degeneration (Pl. 2, fig. 12), except in the case where striatal damage is evident. Here dust-like degeneration is seen outside the internal capsule,

but strictly confined to the area of cellular damage and not extending beyond it. This method, then, affords no evidence of a cortico-striate projection of myelinated fibres.

All the brains stained by the Nauta method and its modifications show a cortico-striate connexion, and are illustrated in Text-figs. 1 to 4. Where both Nauta &

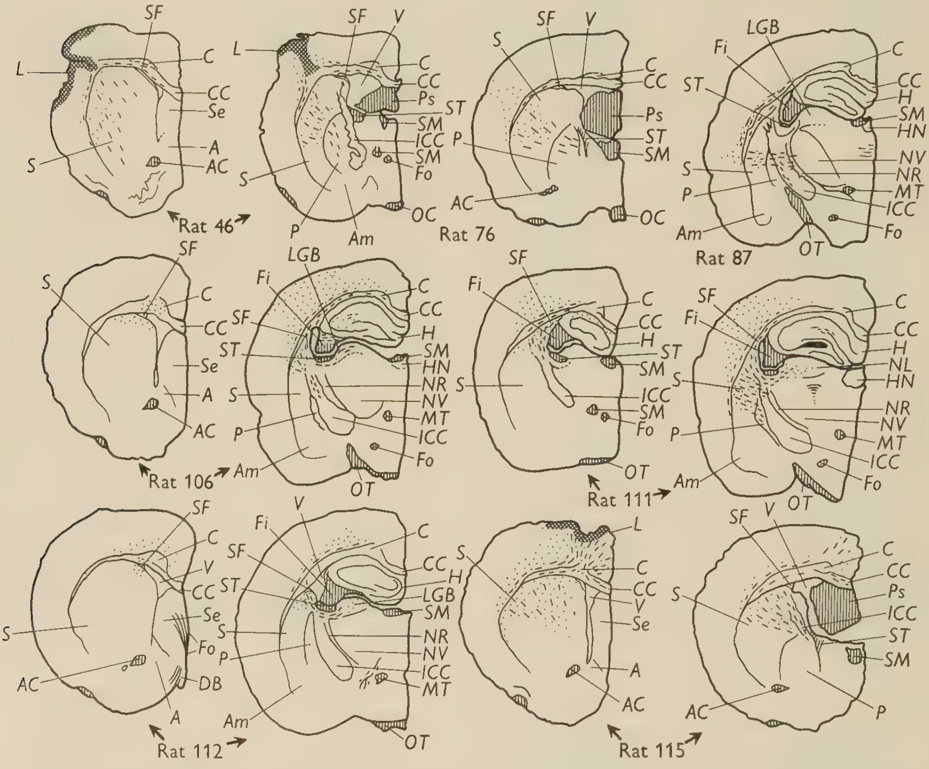


Text-fig. 2. Cortical lesions and striatal degeneration. For explanation see Text-fig. 1.

Gygax (1951, 1954) methods have been used on the same animal there is complete agreement in the defined region of projection.

With cortical lesions situated rostrally, such as those found in rats 1, 86 and 90 (Text-fig. 1), invading the motor areas for the neck, forelimb and snout, degeneration can be traced through the white matter into the homolateral internal capsule and

striatum. The axonal debris lies scattered among the cells in the rostral striatum both rostral and caudal to the anterior commissure. These degenerating fibres are easily distinguished from groups of axons which swing out tangentially from the internal capsule bundles to rejoin the internal capsule more caudally. Changes in the position of the lesion produce changes in the location of the region of the striatum found to contain degeneration. The striatal degeneration is more widespread dorsally than nearer the pallidum, as shown in Text-fig. 4. There is no convincing sign of axons terminating in the pallidum in any of the present material. The pallidum of the



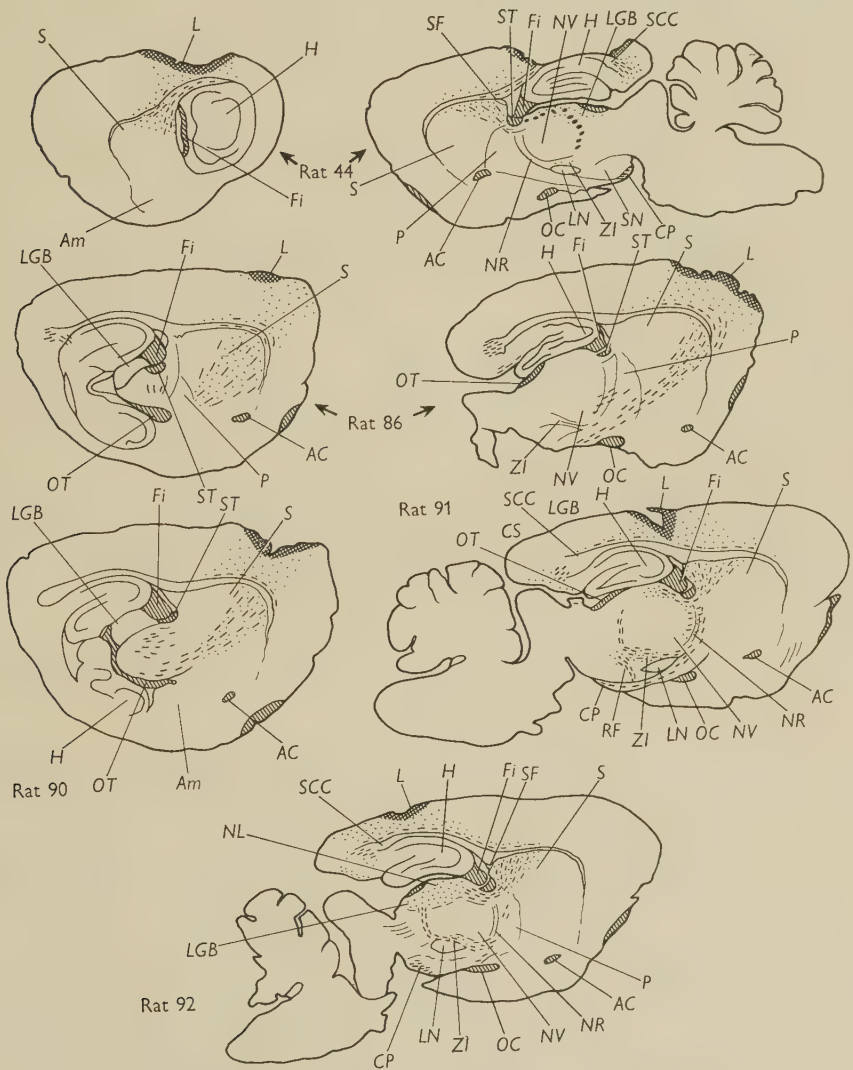
Text-fig. 3. Drawings of sections showing distribution of degenerating axons impregnated with silver. Dashes represent fibres of passage (in bundles or individually), and dots terminal plexuses.

rat, however, is packed densely with internal capsule fibres, making assessment of degeneration difficult.

Lesions in the occipital cortex, as illustrated by rats 44 (Text-fig. 1), 18, 22 and 106 (Text-fig. 2), give rise to degenerating axons which can be traced to the caudal striatum. This distribution at once shows an antero-posterior topography in the projection when compared with the previous examples.

The cortico-striate fibres can be shown to be organized mediolaterally by comparing animals 76, 115 and 86 (Text-fig. 1), and rats 91 and 87 (Text-figs. 1, 2). A progressive lateral shift in the position of the cortical lesion produces a lateral displacement of the region of the striatum containing degenerating axons. In many

cases the degenerating axons leaving the internal capsule to form complex pericellular 'nests' of degeneration in the striatum look very like collaterals (Pl. 2, figs. 6, 7). Rat 91 also defines the region of the striatum receiving fibres from the hind-leg motor area when allowance is made for some damage to the subcortical white matter (cf. rats 92, 44).

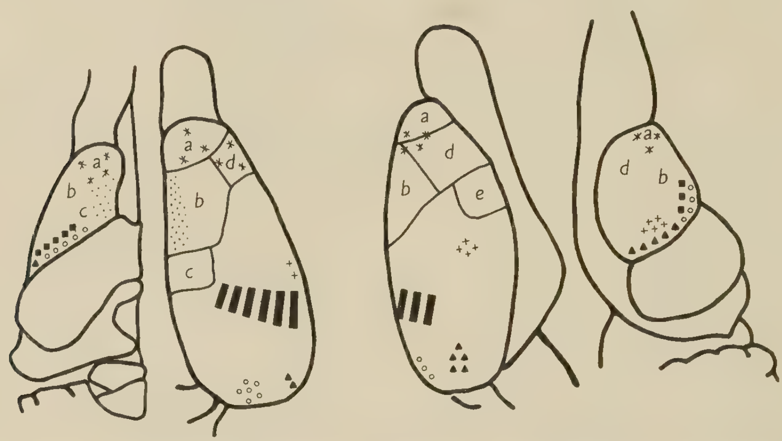


Text-fig. 4. Degeneration plotted on drawings of sections. For explanation see Text-fig. 3.

In the more posterior striatum the mediolateral organization of the projection is distorted by the wedge shape of the caudate-putamen in this region. This is shown by rats 92, 112 and 111 (Text-fig. 2). These occipital lesions lie successively more laterally, but the areas of striatum receiving corticofugal axons lie progressively more posterolaterally rather than laterally.

The lesion in rat 112 lies in the visual area, and invades the white matter only slightly at the centre. The auditory cortex is ablated in rat 111, but with more involvement of subcortical white matter.

In rats 34 and 46 the lesions involve the subcortical white matter to a large extent, and this accounts for the degeneration found in the caudal striatum, since axons from the occipital cortex are interrupted as they turn ventrally at the level of the rostral border of the hippocampus.



Text-fig. 5. Plan of cortico-striate projections in the rat, to summarize the topographical arrangement. Explanation given with Text-fig. 1. The motor regions are modified from Lashley (1941): *a* = neck; *b* = forelimb; *c* = hindlimb; *d* = snout; *e* = mouth and tongue.

Text-fig. 5 summarizes these results, illustrating that the striatum receives fibres from widespread areas of cerebral cortex in a topographically well-organized manner.

Striato-cortical projections

In none of the Golgi preparations has it been possible to trace axons from striatal cells to the cerebral cortex.

Also, in twenty-two brains with cortical ablation no definite changes in striatal cells are seen, unless the striatum is involved by the lesion. Such changes are interpreted as a direct effect of the ablation. In one specimen, however, shrinking and increased basophilia of striatal 'giant' cells confined to the side of the lesion has been noted. The lesion involves only cortex. The significance of this one result is uncertain, and it must be concluded that the investigation fails to demonstrate a striato-cortical projection. This does not exclude the possibility that such a connexion exists.

DISCUSSION

Cortico-striate connexions

Cajal (1891) is the first to have described cortico-striate fibres in the rat. Nauta (1953) reports a connexion from the cingulate cortex of the rat, and Combs (1951) notes rarefaction of the striatal neuropil after hemi-decortication and 4 days survival, concluding that this represents loss of cortico-striate axons. The present investigation

suggests that such rarefaction is due to some effect of a large lesion other than structural damage to cell processes.

Marchi's method fails to demonstrate cortico-striate fibres in the rat, confirming previous reports by Le Gros Clark (1933) and Krieg (1947).

In other species there is much confusion in the results based on Marchi material. For example, Levin (1936), Krieg (1954) and others in monkeys, and Probst (1901, 1903) and others in cats have been unable to find cortico-striate connexions. On the other hand, Polyak (1927) with cats, Coenen (1929) with rabbits, Bianchi (1914), and Mettler (1935*a*, *b*, *c*, 1942, 1945, 1948) in monkeys and cats, all find projections. Mettler (1957) has since been unable to record cortico-caudate impulses and has cast doubt on his former work, and the use of the Nauta & Gygax (1954) stain has not clarified matters. For example, Whitlock & Nauta (1956) find projections from the cortex of all three temporal convolutions to the putamen and tail of the caudate nucleus of the monkey. But De Vito & Smith (1959) also using monkeys, report no degenerating fibres in any part of the corpus striatum following ablations of the supplementary motor area.

There is no relation between the species used and the nature of the results; and it is impossible to account for minor differences of technique. If the projection were from a limited area of cortex then lesions outside this region would yield negative results, and this might explain some of the contradictory reports. However, there is no agreement about the extent of cortex involved in the connexion proposed by various authors. Thus Mettler (1948) has reported that area 9 projects to the head of the caudate nucleus and area 6 to all parts of the corpus striatum; area 4 has no contribution. Hirasawa & Kariya (1936) propose that area 4 takes part in the projection. Dusser de Barenne *et al.* (1942) state that the strip-areas project to the caudate nucleus and areas 4 and 6 to the putamen. More examples are easily found in the literature. The present results also make such an explanation unlikely, since the projection originates from widespread areas of cortex.

Another possible explanation of the confusing reports is that some authors (e.g. Wilson (1914), Spiegel (1919), Vogt & Vogt (1920)) maintain that the degeneration seen in fibre bundles of the striatum following cortical ablation represents corticofugal axons passing through to join the internal capsule, whereas others (e.g. Hirasawa & Kariya (1936), Kato (1938)) interpret the same result as demonstrating a cortico-striate connexion. Mettler's (1942) attempt to substantiate this view is not satisfactory, since the demonstration that fibre bundles in the striatum contain axons arising from both striatal and cortical cells cannot resolve the problem.

In the present work the striatal degeneration has been considered to be terminal because the Nauta & Gygax (1954) method shows complex 'nests' of degenerating axons around striatal cells. The earlier method of Nauta & Gygax (1951) shows a similar arrangement of degenerating, fine, preterminal fibres. Since the degeneration products cannot be stained by Marchi's method, the axons shown by the Nauta techniques must be thinly myelinated, and it seems unlikely that fibres randomly passing through the striatum without synapsing would comprise only the finely myelinated elements. The Golgi preparations show that some, if not all, of the cortico-striate fibres are collaterals of axons in the internal capsule (Cajal, 1891).

The exact details of termination are obscure. Glees (1944) notes that cortico-

striate fibres in the cat have no boutons terminaux. This is confirmed in normal material stained by silver methods by Bielschowsky (1919) and in the present investigation. Boycott, Gray & Guillery (1960) propose that the absence of rings of neurofilaments, identifiable by electron microscopy in some synaptic regions, prevents the demonstration of boutons by silver stains, because such stains depend upon the impregnation of neurofilaments. This explanation may well apply to the striatum.

The topographical organization described in this work is of interest in view of Forman & Ward's (1957) ability, with implanted electrodes, to elicit movements by stimulating the head of the caudate nucleus of the cat, and to show topographical representation of head and neck, forelimb and hindlimb. No previous authors have described the cortico-striate projection as topographically organized in both mediolateral and anteroposterior planes. Dusser de Barenne *et al.* (1942) find an anteroposterior but not a mediolateral organization. Whitlock & Nauta (1956) indicate in their descriptions of temporal lobe projections to the putamen and tail of caudate nucleus some mediolateral organization, but are not precise on the point.

The existence of a connexion between the cortex and striatum has been proposed by some workers to explain the participation of the striato-pallidum in inhibitory and facilitatory phenomena elicited by stimulating the cortex (e.g. Dusser de Barenne *et al.* 1942). To pass an opinion on the differential projection proposed by those using the strychnine method is not possible, because in the rat the striatum is not divided into caudate nucleus and putamen. The problem is being investigated in the cat. The exact significance of a collateral projection as shown here remains obscure. The corpus striatum may play a more important part in the performance of movements than previously supposed, and the widespread source of its cortical afferents is possibly an indication of this importance.

Striato-cortical connexions

The present failure to produce retrograde cell changes in the striatum is in agreement with the majority of previous results. Holmes (1901), Morrison (1929) and others have been unable to find such changes in cats and dogs. Ferraro (1924) finds cellular changes if the lesion involves the caudate nucleus. Minkowski (1923) describes fibre loss in the putamen and caudate nucleus of the monkey immediately deep to cortical lesions but cell changes in the putamen and pallidum only. Kodama (1927) reports changes only in the pallidal cells after cortical ablation. The present findings agree exactly with the observations of Ranson, Ranson & Ranson (1941) on the brain of a partially decorticate monkey.

The work of Mettler, Grundfest & Hovde (1952) and Hovde & Mettler (1953) recording short-latency striato-cortical impulses has been confirmed, e.g. by Purpura, Housepian & Grundfest (1958). However, the conclusion that cell counts reveal a loss of striatal cells after cortical ablation in monkeys still awaits confirmation (Harman, Tankard, Hovde & Mettler, 1954). Previously Mettler (1943) has maintained striatal shrinkage after cortical ablation to be due to loss of cortico-striate neuropil, because cell counts show the density of cell-packing to have increased.

Whilst the present material affords no obvious histological evidence of a striato-cortical projection it remains possible that counting would reveal cell loss. Also,

negative evidence from retrograde-change experiments must be accepted with reservations, e.g. lesions in the fornix do not result in shrinkage of hippocampal cells (Daitz & Powell, 1954). A striato-cortical projection in the form of collaterals would account for the absence of retrograde degeneration in the striatal cells, whilst providing a basis for the electro-physiological results. Such a projection has been proposed from the giant efferent cells of the striatum (Cajal, 1895) but awaits confirmation.

SUMMARY

1. Interconnexions between the cerebral cortex and striatum of the rat have been investigated.

2. The cortex projects to the homolateral striatum by fibres that are probably thinly myelinated collaterals of internal capsule axons. They do not appear to terminate in argyrophilic end-feet.

3. The projection is topographically well organized in both anteroposterior and mediolateral planes, and is derived from widespread areas of cortex, including probably the auditory and visual areas.

4. No evidence of a cortico-pallidal projection has been found, but the problem requires further investigation.

5. It has not been possible to produce convincing evidence of true retrograde changes in the striatum after cortical ablation.

I should like to thank Prof. J. Z. Young, F.R.S., for his stimulating criticism and advice during the course of this research, and also acknowledge kind help with the numerous difficulties from the late D. A. Sholl.

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ABBREVIATIONS

A	Nucleus accumbens	*NL*	Lateral thalamic nucleus
AC	Anterior commissure	*NR*	Reticular nucleus of thalamus
Al	Alveus	*NV*	Ventral thalamic nucleus
Am	Amygdaloid complex	*OC*	Optic chiasma
C	Cingulum bundle	*Olf*	Olfactory bulb
CC	Corpus callosum	*OT*	Optic tract
CP	Cerebral peduncle	*P*	Pallidum
CS	Superior colliculus	*Ps*	Hippocampal commissure
DB	Diagonal band of Broca	*RF*	Radiations of Forel
Fi	Fimbria	*S*	Striatum
Fo	Fornix	*SCC*	Splenium corpus callosi
H	Hippocampus	*Se*	Septum
HN	Habenular complex	*SF*	Subcallosal fasciculus
IC	Internal capsule bundle	*SM*	Stria medullares
ICC	Compact part of internal capsule	*SN*	Substantia nigra
L	Lesion	*ST*	Stria terminalis
LGB	Lateral geniculate body	*V*	Lateral ventricle
LN	Subthalamic nucleus	*W*	Subcortical white matter
MT	Mammillo-thalamic tract	*Z*	Zona incerta

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Low-magnification view of a parasagittal section from *rat* 92, with a lesion in the posterior cerebral cortex. Degeneration is confined to the posterior striatum. Nauta & Gygax (1954) stain.

Fig. 2. Detail from postero-superior region of striatum of *rat* 92, above.

Fig. 3. Control for *rat* 92, above. Similar region of the striatum of an unoperated brain stained in the same way as *rat* 92.

Fig. 4. Rat striatum, showing collaterals (*C*) leaving internal capsule axons (*A*) and entering the striatum. Golgi rapid method.

Fig. 5. A collateral (*C*), leaving an internal capsule axon (*A*), and bifurcating in the striatum. Golgi rapid method.

PLATE 2

Fig. 6. *Rat* 112, lesion in visual cortex. Detail from posterior striatum, showing that degenerating axons leaving the internal capsule have the appearance of collaterals. Nauta & Gygax (1954) stain.

Fig. 7. Collateral degeneration leaving an internal capsule bundle and entering the striatum to form pericellular 'nests'. *Rat* 91. Nauta & Gygax (1954) method.

Fig. 8. Control for *rat* 112, Fig. 6. Posterior striatum of an unoperated brain.

Fig. 9. Control for *rat* 91, Fig. 7, from the striatum of a normal brain.

Fig. 10. Posterior striatum in *rat* 106: fine, degenerating axons stained near their termination are arrowed. Oil immersion. Nauta & Gygax (1951) stain.

Fig. 11. Control for *rat* 106, Fig. 10. Striatum of unoperated brain.

Fig. 12. The internal capsule bundle on the left contains degenerating axons, but the striatum is empty of degeneration. Compare with Figs. 6 and 7. Swank & Davenport Marchi method.

Plate 3

Fig. 13. *Rat* 54, showing normal appearance of striatum on unoperated side. Nissl stain.

Fig. 14. *Rat* 54, normal striatal fibre plexus. Peters's stain.

Fig. 15. Striatum subjacent to lesion in *rat* 54. Note the shrunken cells (compare Fig. 16). (Deeper parts of striatum are normal.) Nissl stain.

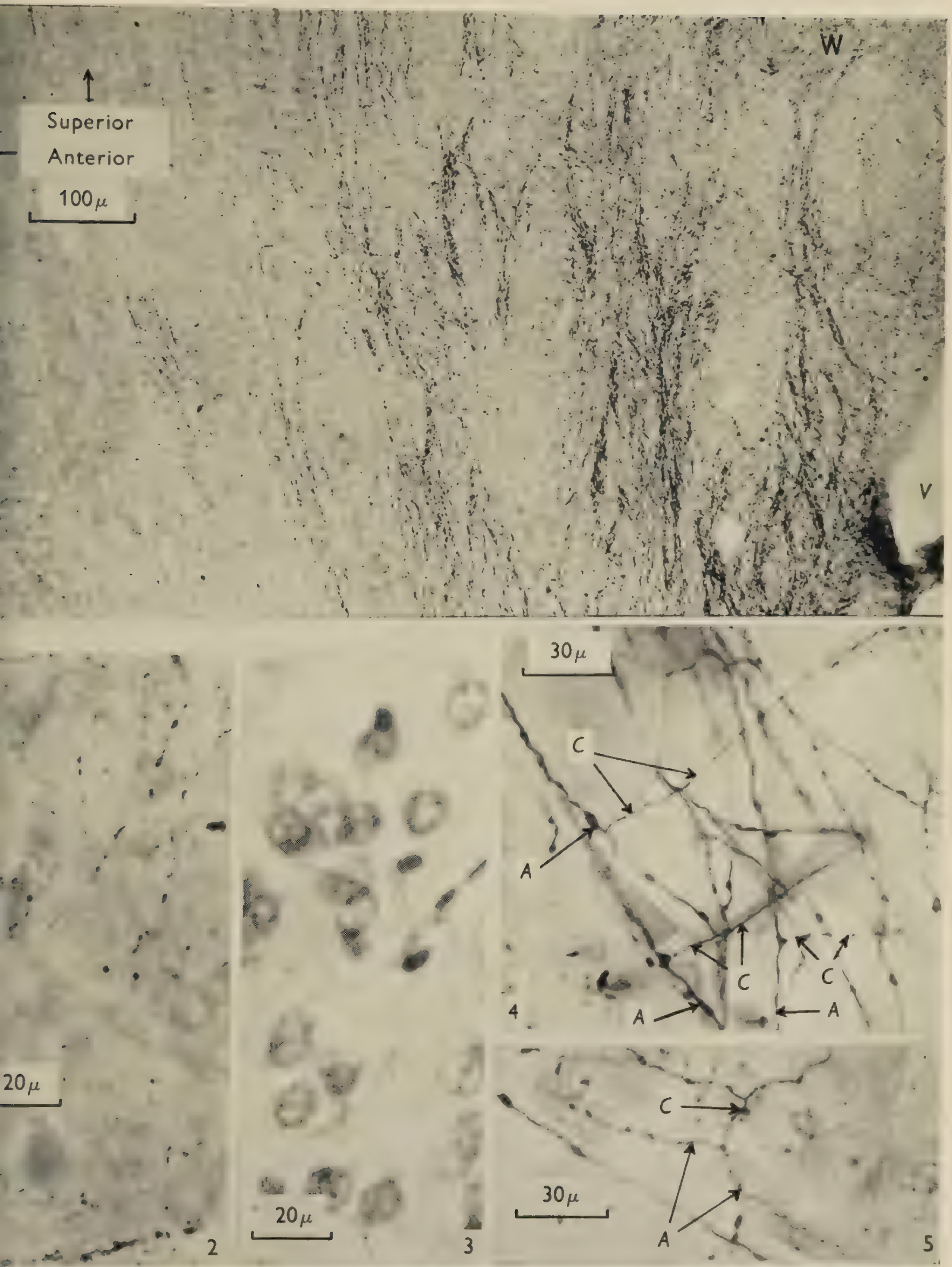
Fig. 16. Disorganization of the striatal neuropil immediately subjacent to lesion in *rat* 54. Peters's stain.

Fig. 17. Showing lack of obvious striatal cell changes after 12 days' survival using the Brodal-Gudden technique. Nissl stain.

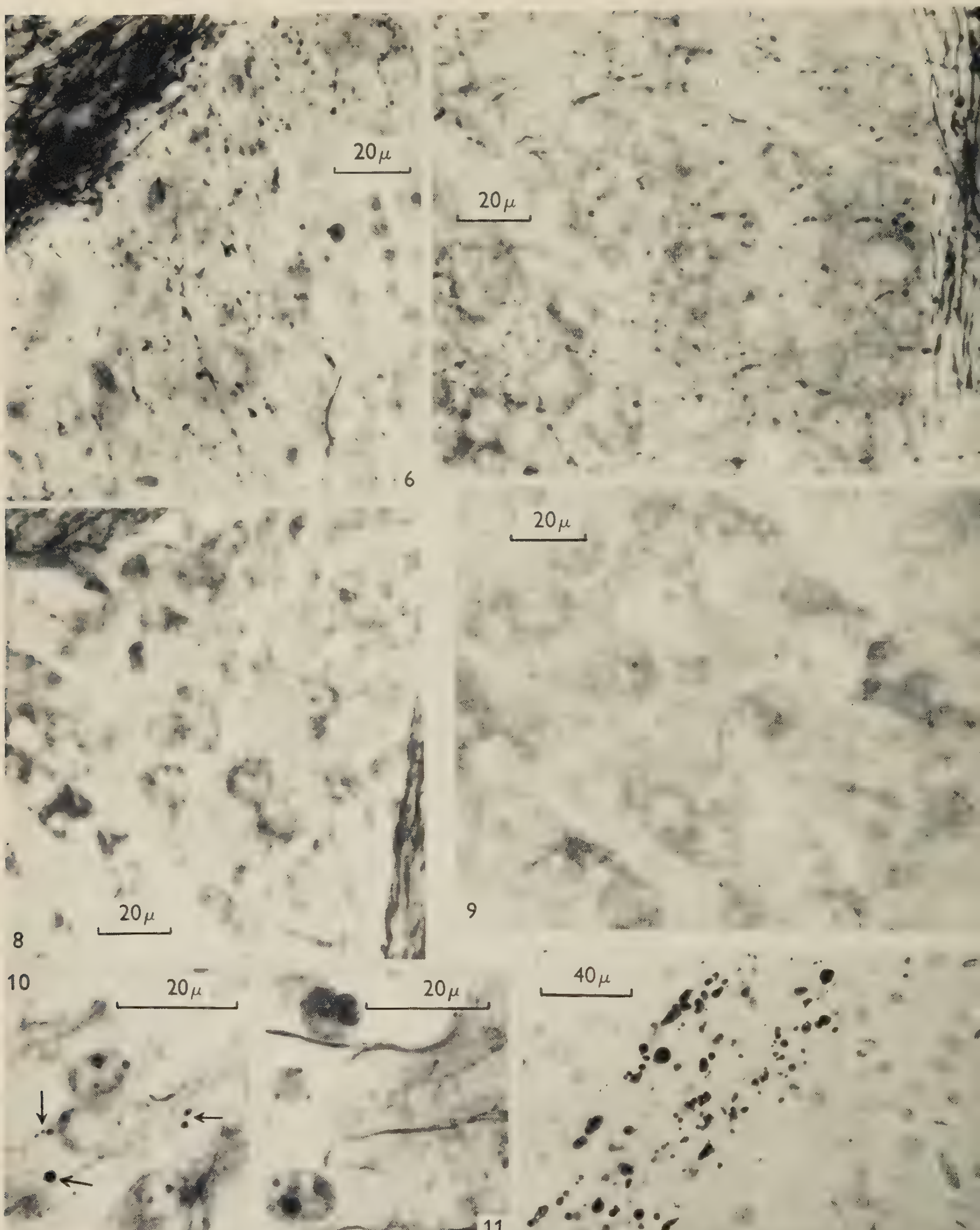
Fig. 18. Control for Fig. 20. Nissl stain.

Fig. 19. *Rat* 56: striatum on side of lesion, showing shrunken 'giant' cells (arrowed). Nissl stain.

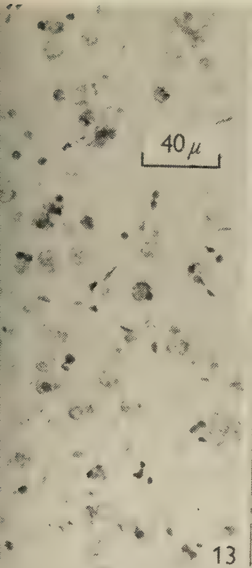
Fig. 20. Control for Fig. 22, illustrating appearance of striatum of opposite side. The 'giant' cells are arrowed. Nissl stain.



WEBSTER—CORTICO-STRIATE INTERRELATIONS IN THE ALBINO RAT



WEBSTER—Cortico-striate interrelations in the albino rat



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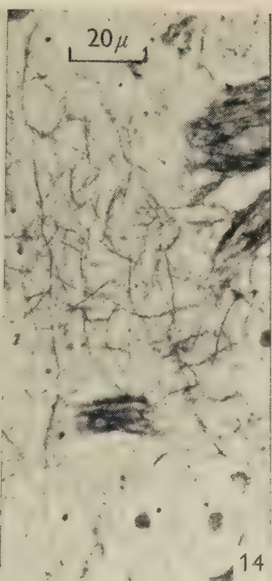

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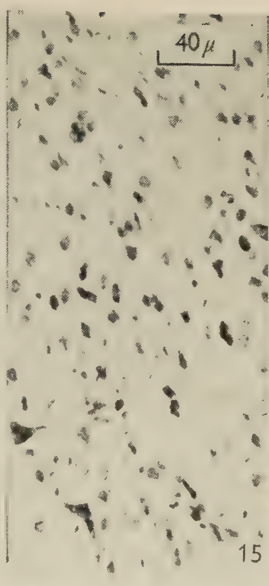

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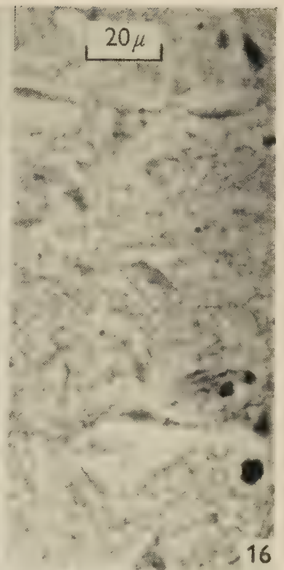

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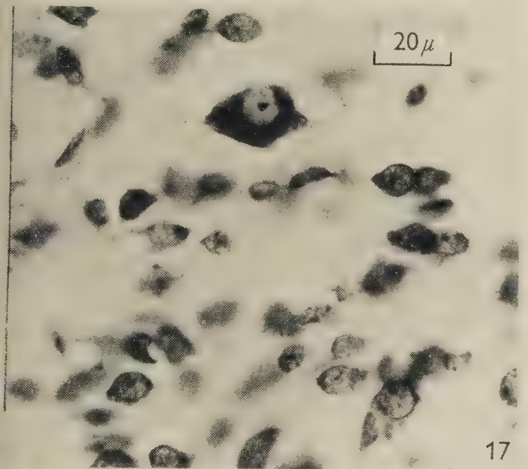

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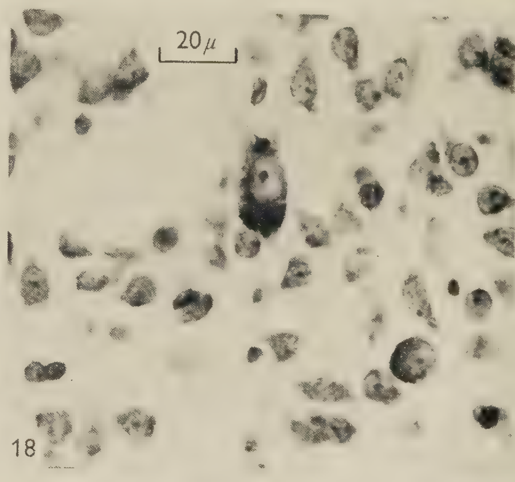

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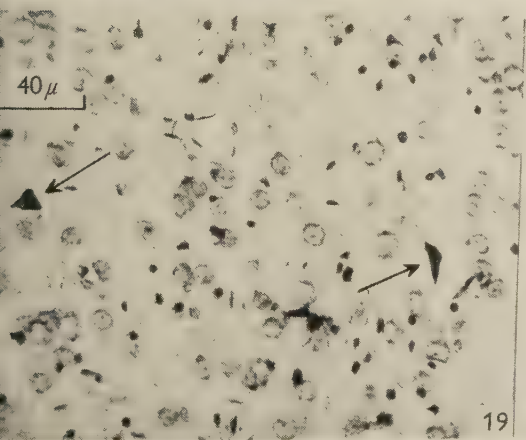

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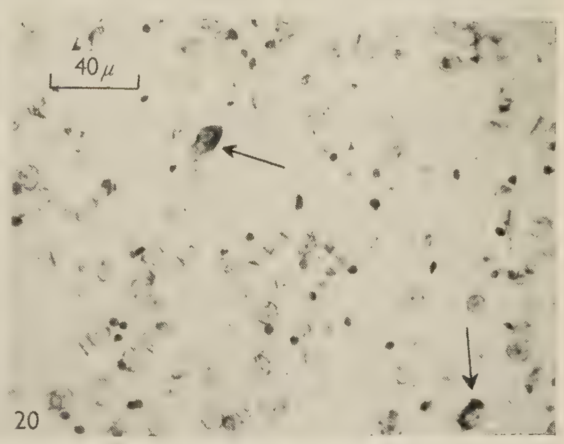

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WEBSTER—CORTICO-STRIATE INTERRELATIONS IN THE ALBINO RAT

AN EXPERIMENTAL STUDY OF THE AVIAN VISUAL SYSTEM

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INTRODUCTION

In a recent experimental study of the thalamic projection upon the telencephalon in the pigeon (Powell & Cowan, 1961), an unexpected finding after telencephalic lesions was the absence of any retrograde cell degeneration in the diencephalic nuclei said to receive afferent fibres from the retina. Taken by itself this finding need not be viewed as evidence against a telencephalic projection in the avian visual system because the pathway from these relay nuclei to the telencephalon may be multisynaptic or their cells may be resistant to axonal section. In accord with the first of these alternative explanations are the electrophysiological observations of Bremer, Dow & Morruzzi (1939) who recorded a marked 'on-response' from the cerebral cortex in the pigeon on illumination of the contralateral eye.

Previous studies of the avian visual system have been made either on normal material or on material stained to show degenerating myelinated fibres (Kappers, Huber & Crosby, 1936). To exclude the possibility of a visual projection to other diencephalic areas, and in particular to those nuclei which have a telencephalic projection, it is desirable that the visual pathway be re-examined with the recently developed axonal degeneration techniques which have been used so successfully in the mammalian and reptilian brains. Until this has been done it must remain uncertain whether the avian visual system more closely resembles that of the mammal or that of the lizard in which there does not appear to be a direct diencephalic relay to the telencephalon (Armstrong, 1950; Powell & Kruger, 1960). That the silver degeneration techniques can be applied to the avian brain has been shown by the comparative study of Evans & Hamlyn (1956) on the relative advantages of the Glees and Nauta techniques. Their finding that axonal and terminal degeneration was as marked in the avian brain as in the mammal also suggested that the avian visual system might provide valuable material for examining the usefulness of other degeneration techniques, in particular of paraffin 'on-the-slide' methods.

In the present study the course and termination of optic nerve fibres has been investigated in the pigeon after unilateral enucleation of the eye using a number of silver methods. In addition we have paid particular attention to the question of centrifugal fibres to the retina and to the possibility of a direct projection to the hypothalamus from the visual system which might be concerned with the regulation of endocrine activity.

MATERIAL AND METHODS

The brains of 14 pigeons were used in this study. In 11 of these unilateral eye enucleation was performed under ether anaesthesia, and, with one exception, the birds were allowed to survive for periods ranging from 5 to 26 days; these brains were fixed by immersion in 10% formal saline. The remaining pigeon survived for 60 days and its brain was fixed in 70% alcohol and 2% acetic acid. Two normal brains fixed in 10% formal saline were used as controls. In addition a number of other brains with no involvement of the visual pathway which had been stained with thionine and Bodian's protargol method were available for comparison.

Table 1

Experiment	Survival period (days)	Plane of section	Preparation
		A. Frozen section methods	
AP 5	5	Coronal	Glees, Bodian, Nauta & Gygax (1954), cresyl violet
AP 6	8	Coronal	Glees, Bodian, Nauta & Gygax (1954), cresyl violet
AP 3	12	Coronal	Glees, Bodian, Nauta & Gygax (1954), cresyl violet
AP 4	15	Coronal	Glees, Bodian, Nauta & Gygax (1954), cresyl violet
AP 1	18	Coronal	Glees, Bodian, Nauta & Gygax (1954), cresyl violet
AP 7	22	Coronal	Glees, Bodian, Nauta & Gygax (1954), cresyl violet
AP 2	26	Coronal	Glees, Bodian, Nauta & Gygax (1954), cresyl violet
AP N	Normal control		Glees, Bodian, Nauta & Gygax (1954), cresyl violet
		B. Paraffin methods	
AP 3 A	12	Horizontal	Marsland, Glees & Erikson, Bodian, Guillery *et al.*, Nauta & Gygax (1951), thionine
AP 1 A	18	Coronal	Marsland, Glees & Erikson, Bodian, Guillery *et al.*, Nauta & Gygax (1951), thionine
AP 2 A	26	Horizontal	Marsland, Glees & Erikson, Bodian, Guillery *et al.*, Nauta & Gygax (1951), thionine
AP N 1	Normal control	Coronal	Marsland, Glees & Erikson, Bodian, Guillery *et al.*, Nauta & Gygax (1951), thionine
EP 1	60	Coronal	Bodian, thionine

Most of the brains were sectioned on a freezing microtome at 12μ or 25μ. The sections were collected in groups of 4 and from each brain a 1 in 16 series, stained according to the Nauta & Gygax (1954) technique, and a second series with the Glees (1946) technique. Two additional series were mounted on gelatinized slides and stained with cresyl violet or, after a coating with thin celloidin, according to the Bodian method. Concurrent with each experimental series a corresponding 1 in 32 series of normal sections were mounted and stained. Three brains were embedded in paraffin wax and cut at 15μ; in each case a 1 in 10 series was stained according to the original Nauta & Gygax (1951) technique, the recent modification of this technique by Guillery, Shirra & Webster (1960), the method described by Marsland, Glees & Erikson (1954), Bodian's (1936) protargol method, and thionine. The alcohol fixed brain was embedded in paraffin wax, sectioned at 25μ and stained with thionine and protargol. The details of the preparation of the 11 experimental and 2 control brains are set out in Table 1.

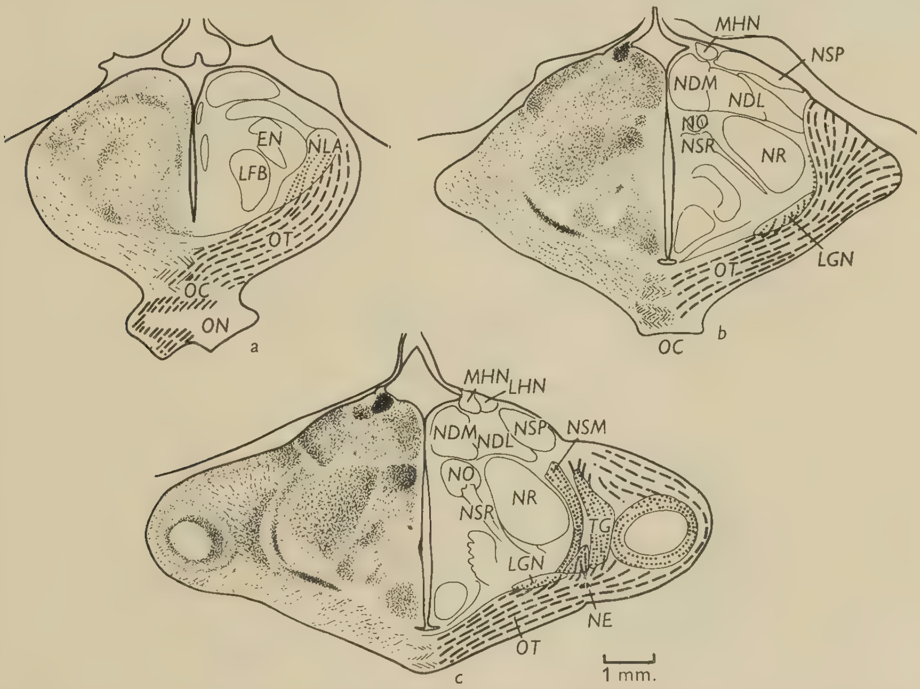
RESULTS

Apart from differences in the degree of axonal and terminal degeneration depending upon the survival period, the experiments are remarkably uniform in their results. For the description of the visual pathway, therefore, only one experiment of

moderate survival period need be described in detail. The differences found after differing survival periods and with the various techniques will be described in a following section.

The visual pathway in the pigeon brain

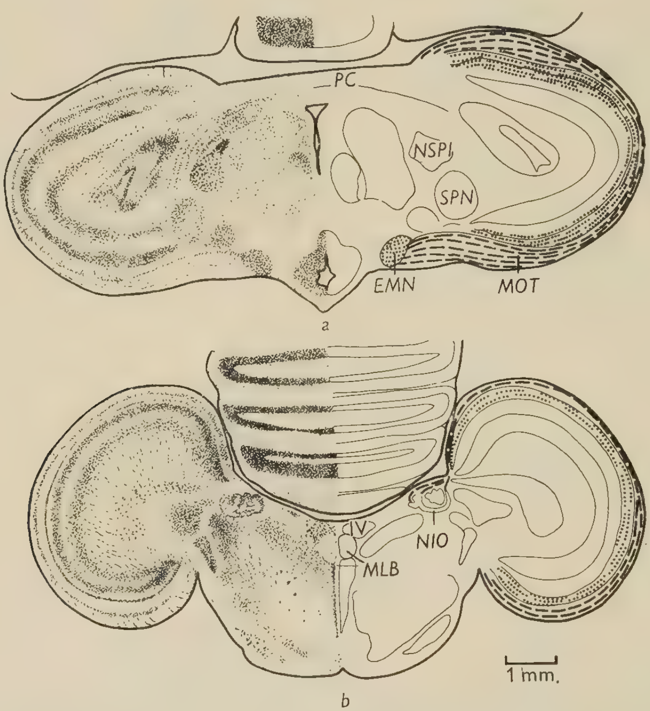
The course and termination of the fibre degeneration will be described as seen in successive antero-posterior sections cut in a coronal plane. The description of the course of the degenerating fibres is based on Nauta-stained sections, and the



Text-fig. 1. The disposition of the principal nuclei of the diencephalon and the related visual pathways at three antero-posterior levels (*a*, *b*, *c*). The cell masses are shown semi-diagrammatically on the left and in outline on the right; the sites of fibre degeneration (broken lines) and terminal degeneration (dots) are also shown on the right. The outlines in this and the subsequent figures were drawn with the aid of a low-power projection apparatus at an original magnification of 20 ×.

terminal degeneration upon Glees and Bodian preparations. Following enucleation of the right eye the optic nerve is severely degenerated throughout its cross-sectional area, and this degeneration can be traced through the optic chiasma where the fibres undergo complete decussation. While it is not feasible to exclude the possibility that a few fibres continue into the optic tract of the ipsilateral side careful examination of all our experimental material, and of the control sections from the normals brains, has failed to show any appreciable number of degenerating fibres on the ipsilateral side, and certainly there is no fasciculus as Armstrong (1950) has described in the lizard brain. The characteristic pattern of normal and degenerating fibres in the optic chiasma is shown in Pl. 1, fig. 2.

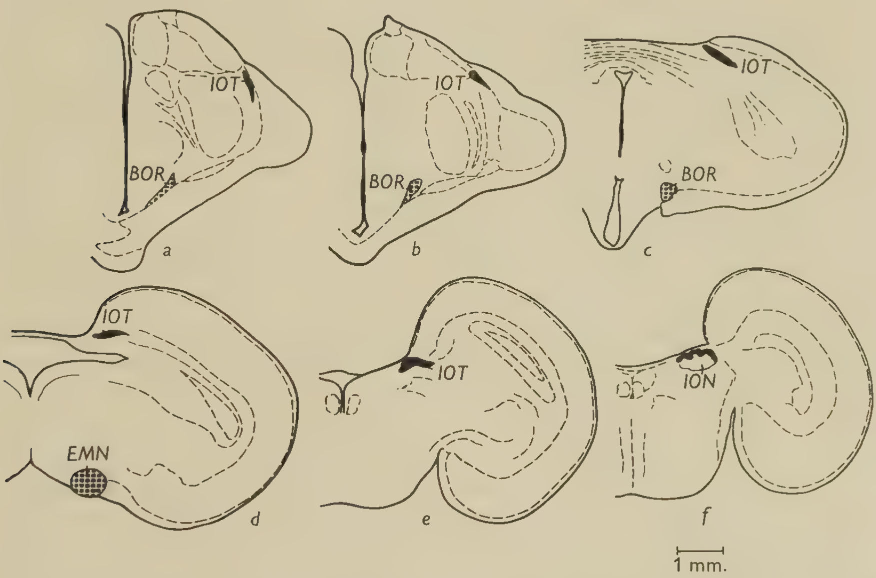
The degenerating fibres pass backwards and laterally into the optic tract, the ventral two-thirds of which is completely degenerated; in the dorsal third, adjacent to the ventral surface of the brain, the amount of degeneration is much less. In under-suppressed material a considerable number of normal fibres are seen in this dorsal part. More posteriorly, in what is known as the marginal optic tract these normal fibres form a distinct bundle along the ventral aspect of the brain and although they can be traced anteriorly into the region of the optic chiasma it has not been possible to determine their further course. The corresponding area in the ipsilateral marginal optic tract contains no degenerating fibres.



Text-fig. 2. Drawings of the nuclei associated with the visual projection at two representative levels (*a*) through the ectomammillary nucleus and (*b*) through the isthmo-optic nucleus at the level of the IVth nerve nucleus.

At the level of the anterior end of the thalamus a diffuse bundle of fibres sweeps dorsally and laterally above and in front of the lateral geniculate nucleus and below the lateral forebrain bundle towards the nucleus lateralis anterior. As it approaches this nucleus the fibres fan out and penetrate it from all aspects except posteriorly and laterally. Within the nucleus preterminal fibre degeneration is seen throughout its extent, and in the Glees and Bodian preparations numerous ring-like boutons are found around many of the cells; in the lateral geniculate nucleus of the opposite side only an occasional bouton can be seen. No degeneration is seen dorsal or posterior to the nucleus indicating that this bundle is terminating only in this nucleus (Text-fig. 1*a*).

More posteriorly, numerous fibres are seen turning dorsally at right angles from the tract to enter the overlying lateral geniculate nucleus (Text-fig. 1*b*, *c*). Within this nucleus most of the fibre degeneration is confined to the ventral part of the nucleus which contains only a few scattered small cells. However, most of the degenerating boutons, shown by the Glees and Bodian techniques, are found amongst the cells of the dorsal part of the nucleus. The degeneration in the lateral geniculate nucleus remains the same throughout its antero-posterior extent. In addition to the fibres which are terminating in the nucleus many degenerating coarse fibres pass through and along the dorsal aspect of the nucleus. Laterally these fibres unite with other large fibres from the optic tract to form the axillary or isthmo-optic bundle.



Text-fig. 3. Tracings at successive antero-posterior levels to show the course and relations of the basal optic root (stippled) and the isthmo-optic tract (solid black).

At the level of the anterior end of the optic tectum a considerable number of fibres are given off at right angles to the tract in the region of the nucleus externus and the so-called tectal grey. In addition to dense preterminal degeneration within these cell masses (see Pl. 1, fig. 4) distinct bundles of fibres can be seen passing dorsally along their medial and lateral aspects to reach the nucleus superficialis synencephali and the deeper parts of the tectal grey (Text-fig. 1*c*). With the appropriate stains numerous degenerating boutons are found in all these nuclei, and they are exceptionally dense in the nucleus externus. For the sake of convenience, it may be pointed out here that the dorsal part of the tectal grey also receives numerous degenerating fibres descending from the dorsal part of the marginal optic tract.

Just behind the level of the optic chiasma distinctly coarse fibres collect on the dorso-medial aspect of the optic chiasma to form the basal optic root (stippled in

Text-fig. 3). More posteriorly this forms a wedge-shaped bundle which passes backwards close to the medial edge of the optic tract to reach the ectomammillary nucleus which is filled with fragmented fibres. Although few boutons in the form of rings are seen, many large, solid end-bulbs are found in close proximity to the cells (Pl. 2, figs. 8, 9).

The isthmo-optic tract first appears as a distinct bundle in the region between the tectal grey and the nucleus rotundus. It arches dorsally and backwards beneath the dorsal margin of the optic tectum and then turns ventro-medially to reach the nucleus of the same name. Approaching the nucleus from its dorso-lateral aspect the fibres spread out to surround all but its ventral surface (Text-fig. 3). Although the fibres of the tract are fragmented right up to, and clearly outline, the nucleus, no fibre degeneration is seen within the limits of the nucleus or among the cells. Similarly, in neither the Glees nor the Bodian preparations is there any evidence of terminal degeneration within the nucleus, although again the fibres of the tract are clearly degenerated. The interpretation of these findings will be discussed later, but it may be noted that in no experiment, nor with any technique, is there any sign of terminal degeneration within the nucleus, and only after a survival period of 26 days is any change found in the neuropil of the nucleus.

Although a careful search was made throughout the diencephalon no evidence of terminal degeneration was found in any nuclei apart from those already mentioned. In particular, no projection of optic nerve fibres could be traced to the hypothalamus or to the nucleus rotundus and pretectal nuclei of the thalamus.

The principal part of the visual projection is undoubtedly to the optic tectum. The marginal optic tract, on reaching the antero-ventral aspect of the tectum, spreads out over its entire surface so that the fibres of the tract come to form the outer layer or stratum opticum of the tectum. Before describing the distribution of degeneration it is necessary to comment upon the terminology used in describing the layers of the optic tectum. The basis of most recent accounts of this region is the comprehensive study of Huber & Crosby (1933) on the reptilian tectum. Their subdivision into six primary layers has been followed by Jungherr (1945) whose description of the chicken tectum we have found to be sufficiently similar to that of the pigeon as not to warrant a separate description of our material (see Pl. 1, fig. 1). These layers are, from without inwards:

(1) stratum opticum;
(2) stratum griseum et fibrosum superficiale;
(3) stratum griseum centrale;
(4) stratum album centrale;
(5) stratum griseum periventriculare;
(6) stratum fibrosum periventriculare.

As no terminal degeneration is found deeper than the second of these layers no further reference will be made to the deeper layers. In the interpretation of the degeneration it is necessary, however, to further subdivide the stratum griseum et fibrosum superficiale. In Nissl-stained perparations (Pl. 1, fig. 1) the following layers can be recognized.

(*a*) a narrow layer of small cells;
(*b*) a slightly thicker, relatively cell-free layer;

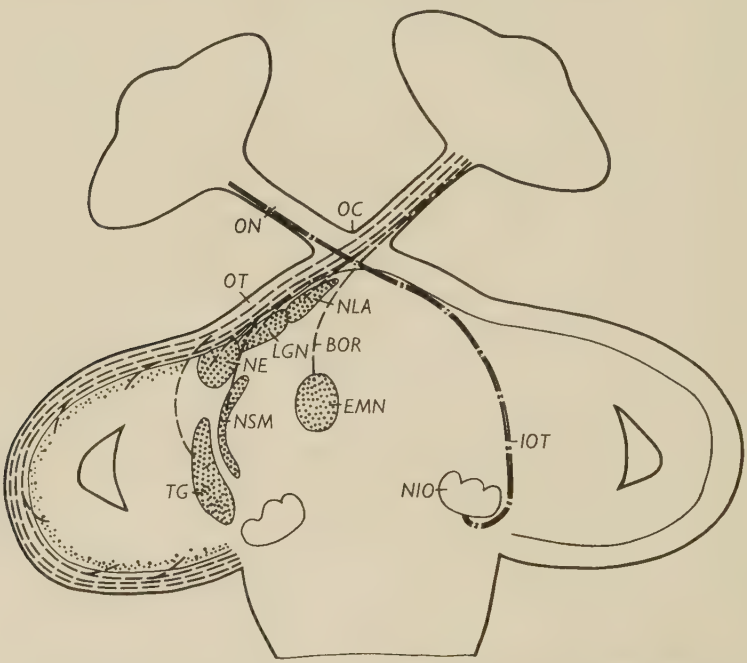
(*c*) a layer of medium and small-sized cells;
(*d*) a second almost cell-free layer;
(*e*) a single layer of medium-sized, pale-staining cells;
(*f*) a narrow cell poor layer;
(*g*) a very narrow layer of deeply staining and compactly arranged cells;
(*h*) a broad layer containing cells of variable size and occasional pyramidal neurons;
(*i*) a thicker layer of more compactly grouped cells of uniform size;
(*j*) a cell poor layer.

The only difference between this description and that of Jungherr (1945) is that the latter makes no mention of the outermost layer of small cells—layer (*a*).

In the experimental material the superficial part of the stratum opticum shows marked fragmentation of all the optic nerve fibres, but in the deepest part, adjacent to the stratum griseum et fibrosum superficiale, there is a considerable number of normal fibres remaining. The number of these normal fibres appears to increase further rostrally in the marginal optic tract. From the stratum opticum fibres enter the stratum griseum et fibrosum superficiale at an acute angle and ramify in its outer part, the appearance of the degeneration varying in the different laminae of this zone. In layers (*a*) and (*b*) the degenerating fibres form an irregular plexus, which appears to be most dense in the more superficial layer in which many of the fibres can be seen to run horizontally for some distance and to break up into fragments. There are comparatively few degenerating fibres in layer (*c*), but in laminae (*d*) and (*e*) distinct degenerative changes have occurred in the radially orientated fibres which form such a prominent feature of these layers in normal material. These fibres are nearly all broken up into isolated fragments but retain their radial arrangement. In the Glees preparations there is definite fragmentation of the fibres in the optic nerve layer, and in the immediately subjacent layer there are a number of degenerating fibres scattered irregularly amongst the normal fibres together with a moderate number of degenerating boutons. In layer (*b*) of the stratum griseum et fibrosum superficiale there are slightly more boutons than in (*a*), but apart from this the fibre plexus shows no change. The fragmented fibres in layer (*c*) are appreciably darker staining but only an occasional bouton is seen. The radial fibres of layer (*d*) are very irregular in both their course and their depth of staining, and many are coiled. The fibres are also distinctly shorter than on the normal side, and, especially in the deeper part of the lamina, appear to be beaded. Throughout the depth of this layer there is an exceptionally large number of boutons, and these are found mainly in the form of rings of variable staining intensity. Amongst the cells of layer (*e*) solid, deeply staining end-bulbs are present; the majority of these are seen in continuity with a short, deeply staining terminal segment of an axon. In the adjacent molecular layer (*f*) occasional boutons and degenerating terminals are seen. It is interesting that in both these layers the boutons are in continuity with fibres which are orientated either obliquely or parallel to those in the stratum opticum or in many cases curl back on themselves; no fibres have been seen to terminate without turning in one or other of these three ways. No boutons or degenerating fibres are seen deeper than this level.

The appearance of the outer layers of the tectum is essentially the same in the Bodian preparations, with the exception that there is no evidence of fibre fragmentation in the stratum opticum or in layer (*a*). In the other affected layers the only appreciable difference is the absence of beaded fibres in layer (*d*); the other degenerative changes in the layer, however, closely resemble those found in the Glees preparations (Pl. 1, fig. 3).

A common finding in all the material was the greater density of boutons in the lateral part of the tectum as compared with more medial regions. This is of interest in the light of the electrophysiological evidence of Hamdi & Whitteridge (1954) that the central portion of the retina projects to the lateral part of the tectum. To



Text-fig. 4. A schematic diagram to show the main features of the visual projection as determined in this study. The broken lines indicate pathways in which fibre degeneration has been traced and the dotted areas in which terminal degeneration is found. The isthmo-optic nucleus and tract are shown separately on the right for reasons given in the text.

confirm this qualitive impression of bouton density a series of counts of the boutons was done in one experiment. Using a graticule inserted into the eyepiece, all the boutons in an area 0·1 mm.^{2} were counted in the dorso-medial, ventro-medial and lateral sectors of the stratum griseum et fibrosum superficiale respectively. At the medial margins of the tectum the mean counts were 194 and 187, while in the lateral area there were 258 boutons. These counts indicate that in the central projection field of the retina there is an increase of approximately one-third in the number of boutons.

The description of the visual pathway which has been given above is typical of the findings in all the coronally sectioned brains, and is essentially similar to our

findings in horizontal preparations although these show some additional features of interest. First, the course of the fibres to the nucleus lateralis anterior is seen particularly clearly in horizontal sections, and in addition they show that the afferents to the tectal grey enter it from all aspects. Secondly, the course and relations of the basal optic root are seen distinctly because in several sections the tract can be traced throughout its entire antero-posterior extent. Another feature is the greater ease with which the nuclei externus, superficialis synencephali and tectal grey can be differentiated where they lie in the angle between the nucleus rotundus, the lateral geniculate and the tectum. One of the most striking features of the horizontal material is the clarity with which the bundle of normal fibres in the deeper part of the stratum opticum can be traced medially into the region of the optic chiasma.

Having given an account of the course of the visual pathways in the pigeon we shall now describe the differences in appearances of the degenerating nerve fibres and terminals after varying survival periods and as seen in material prepared with several silver impregnation techniques. Subsequently we shall present certain unexpected findings in this material bearing on the question of a possible centrifugal projection to the retina and on the interpretation of axonal degeneration in the nervous system.

Observations on the nature and time course of fibre degeneration

From the point of view of tracing the central connexions of the retina all the techniques used in this study have given essentially similar and equally consistent results. It would have been possible to determine the principal sites of termination of optic afferents with any single technique, but by using most or all of them on the same material (i.e. on successive sections) we have obtained both a clearer picture of the course and mode of fibre termination and also of the nature of the degenerative process.

The relative advantages of the Nauta and Glees methods as commonly used on frozen sections are well known (Glees & Nauta, 1955; Evans & Hamlyn, 1956; Bowsher, Brodal & Walberg, 1960). Thus, while the Nauta technique selectively stains degenerating fibres through the suppression of normal fibres it does not impregnate the finer axonal terminals and boutons; the Glees method, on the other hand, will demonstrate degenerating terminals and boutons in addition to the degenerating fibres, but this degeneration is considerably more difficult to distinguish as the normal fibre plexus is also impregnated. In our hands the technique for paraffin sections described by Marsland *et al.* (1954) gives essentially the same picture as the original Glees method, but the appearance of degeneration and of the normal fibre plexus in sections stained with either the Nauta & Gygax (1951) or the recent modification of the method (Guillery *et al.* 1960) differs in a number of important respects from the frozen section method as now commonly used (Nauta, 1957). Apart from the obvious advantage that serial sections are more easily obtained with paraffin embedded material, the most significant advantage of the latter technique is that it stains fine terminals and boutons as well as giving a considerable suppression of normal fibres. The principal fibre tracts are usually impregnated so that the appearance is more or less intermediate between the

conventional Glees and Nauta techniques. The staining of these fibre bundles is not necessarily disadvantageous; for example, in the thalamus this impregnation of normal fibres serves to delimit clearly many of the nuclei. The only thing that need be said about the use of the Bodian technique is that, while it has not generally been used for experimental purposes to show degenerating terminals, we have found that it stains degenerating boutons with even greater clarity than the Glees method, but is less useful for determining degeneration of fibre tracts.

The differences in appearance at varying survival periods with the Glees and Nauta techniques on frozen sections have already been described for the avian optic tectum by Evans & Hamlyn (1956). Our findings with these methods are in essential agreement with theirs, but it is necessary to describe the appearance of the other optic centres and to give the findings in the material prepared with the paraffin methods we have used. Because the findings in the lateral geniculate nucleus, the nuclei lateralis anterior, externus and synencephali and the tectal grey are basically the same, these nuclei may be grouped together in the following account.

It should be emphasized that very few boutons are seen in normal sections through these areas or on the ipsilateral side in the experimental material with any of the techniques used. As Armstrong (1950) found in the visual system of the lizard, and as is well known in mammalian experimental material, one of the earliest signs of degeneration in these nuclei is the appearance of numerous clear, ring-like boutons. Were it not for their absence in control material these early degenerating boutons would be indistinguishable from 'normal boutons' as commonly described. Subsequently the degenerating rings become thickened, enlarged and finally converted into solid end-bulbs.

The earliest degenerative changes are found in these areas 5 days after eye-enucleation. In the Glees and Bodian preparations a moderate number of boutons are found scattered throughout the nuclei together with increased argyrophilia of the terminal fibres. At this stage the Nauta sections show only an irregular impregnation and spindling of the optic nerve fibres, the control side showing a complete suppression of staining. The changes at 8 days are so little advanced as to require no further comment, but in the two animals which survived 12 days there is an appreciable change in the degree of degeneration. The brain of one of these animals had been embedded in paraffin and sectioned horizontally; in the other, frozen sections were cut in the coronal plane. In the frozen sections stained with the Nauta technique numerous degenerated fibres are seen either in a beaded or fragmented condition. In the lateral geniculate nucleus the degeneration is more conspicuous in the outer plexiform layer and in the tectal grey degeneration is arranged in a definite peri-cellular pattern. In the Glees and Bodian preparations of these sections more degenerating boutons and terminals are seen than at any other survival period. The boutons are in all stages of degeneration from simple rings to solid end-bulbs, many of which are attached to a short segment of a terminal fibre. In addition, in the Glees sections many degenerating fibres are seen with a characteristic beaded and fragmented appearance. The most striking feature of the paraffin Nauta sections through these areas is the presence of a considerable number of degenerating boutons having essentially the same appearance as in the Glees

sections. This terminal degeneration is, of course, in addition to the fragmented fibres which can be traced into and through these areas.

At longer survival periods the number of degenerating boutons progressively decreases until at 22 days only an occasional ring is seen. The fibre degeneration, on the other hand, becomes progressively more severe up to 22 days after which there is an appreciable loss of neuropil in these nuclei.

The much larger myelinated fibres which pass through the basal optic root to reach the ectomammillary nucleus appear to degenerate more rapidly than the optic tract fibres. As early as 5 days after enucleation the Nauta technique on frozen sections shows some fragmentation of the fibres in the basal optic root and scattered argyrophilic droplets in the ectomammillary nucleus. In addition, there is, in and around the nucleus, a considerable number of coarse rings comparable to those described by Evans & Hamlyn (1956) in preparations subjected to 'lipid fixation' prior to treatment with fat solvents. These are quite distinct from the boutons seen in the paraffin Nauta sections after slightly longer survival periods. In the Glees preparations at 5 days there is some increased argyrophilia of the fibres of the basal optic root and in the ectomammillary nucleus there are many ring-like boutons. The Bodian preparations of the tract give no evidence of degeneration but there is an appreciable number of boutons in the nucleus. At 12 days the degeneration in this system has progressed to an advanced degree: all the preparations show a virtually complete break-up of the fibres of the tract into large deeply staining droplets, and in the Bodian material there is an appreciable fibre-loss (see Pl. 2, figs. 6, 7). Longer survival periods show a continuing fibre loss although there is still a good deal of degeneration in both the tract and nucleus even at 26 days.

Our findings on the time course of the degeneration in the optic tectum parallel those of Evans & Hamlyn (1956) and only a few significant differences need be described. The first of these is that the degeneration appears to occur more rapidly in the pigeon than in the chicken. At 5 days the Glees and Bodian preparations show not only an increased argyrophilia in the fibres of the outer layers of the tectum but also numerous boutons, especially in the deeper parts of the affected region. The Nauta degeneration is also fully developed by 12–15 days and the paraffin Nauta sections show a considerable number of rings and drop-like disintegration of the fibres at this stage. With longer survival periods the degeneration becomes more limited to the superficial layers of the tectum, and by 26 days there is a considerable loss of the radially orientated fibres in the stratum griseum et fibrosum superficiale. At this stage the Bodian preparations show a very severe fibre loss in the outer two-thirds of the stratum opticum, but in the deeper part of this layer there are numerous, apparently normal, fibres persisting. The number of these fibres increases in the ventro-medial part of the tectum where they can be traced quite clearly into the marginal optic tract and hence to the region of the optic chiasma. There is no loss of fibres in the comparable region of the ipsilateral tectum.

As mentioned above, no evidence of degenerating boutons or pericellular fibre fragmentation has been seen in the nucleus isthmo-opticus after any survival period or with any of the techniques used but there is some loss of neuropil and break-up of the coarse fibres in the nucleus after 26 days. Furthermore, although the diameter of the fibres in the isthmo-optic tract is slightly greater than those in the optic tract,

the time course of the degeneration is appreciably slower. No degeneration is seen until 12 days and at this stage it is present only in the part of the tract immediately adjacent to the nucleus, and can only be seen in the frozen Nauta sections. By 18 days the whole length of the tract is affected, but apart from the sections close to the nucleus the fibres show only increased argyrophilia and irregularity of outline. Beading of fibres along their whole length is not seen until 22 days and the degeneration is not fully developed until 26 days. These findings present a paradox: after short survival periods there is distinct degeneration in the tract close to the nucleus, and although the degenerating fibres surround the nucleus on all but its ventromedial aspect, no degenerating fibres or boutons can be seen within the nucleus. On the other hand, after long survival periods when the tract is degenerate throughout its extent slight fragmentation of fibres is seen within the nucleus but with no evidence of boutons (Pl. 3, figs. 12, 13). A second distinctive feature of degeneration in this nucleus is the fact that only here do cellular changes occur after eye enucleation. In thionin-stained preparations shrinkage and pallor of the cells has been found after 18 days. At 26 days this neuronal atrophy is more severe: the nucleus as a whole is smaller and there is appreciable gliosis throughout the nucleus. After 2 months severe cell-loss has occurred and the surviving cells are shrunken and poorly stained (Pl. 4, figs. 14–16). The possible interpretation of these findings will be discussed later.

One interesting finding in this material will be described although it has no direct bearing on the central projection of the retina but is relevant to the interpretation of the isthmo-optic tract. In all experiments with survival periods of 12 days or longer unequivocal fibre degeneration has been found in the IVth cranial nerve on the affected side. At 12 days the proximal part of the nerve from the dorso-lateral aspect of the nucleus to the decussation on the dorsum of the midbrain shows distinct axonal break-up. In the trochlear decussation and in the more distant parts of the nerve the axons appear completely normal. By 18 days the degeneration has extended into the decussation and here the intermingling of the degenerating axons with the normal fibres from the opposite side can be clearly seen. By 26 days the degeneration has extended into the nerve where it lies on the lateral aspect of the midbrain (Pl. 3, figs. 10, 11). This description of degeneration in the IVth nerve applies only to material prepared with the Nauta technique (frozen and paraffin). In adjacent sections stained with the Glees and Bodian methods no changes were found until 26 days when the Glees sections show increased argyrophilia and irregularity of the degenerating axons. Even at this stage the Bodian technique shows only slight narrowing and varicosity of the fibres. Although no fibre degeneration was seen within the IVth nerve nucleus at any stage between 15 and 26 days, the cells of this nucleus showed characteristic chromatolytic changes; by 2 months these cells have fully recovered their normal appearance. Similar cellular and fibre changes have been seen in the IIIrd cranial nerve but because of the bilateral distribution of the fibres in this nerve these changes have been more difficult to assess; no statement will be made about the VIth nerve as the plane of section of most of the material did not permit of a systematic study of the lower brain stem.

DISCUSSION

The account given of the central projection of the optic nerve fibres in this article is in close agreement with descriptions in the older literature based upon the study of normal and Marchi-stained material (Huber & Crosby, 1929). Although a variety of silver techniques has been used in this study almost any single technique would have proved adequate for tracing the central connexions of the retina, and either paraffin or frozen sections could have been prepared. Each technique has, of course, certain advantages and disadvantages for a study of this type. For example, in the Nauta & Gygax method (1951) and in the modification of this technique by Guillery *et al.* (1960), not only are the degenerating fibres clearly shown but the terminal boutons are also impregnated. On the other hand, while degenerating boutons are shown as clearly with the Bodian technique as with any of the others, it is much less useful than the latter for tracing degenerating fibres.

Taken together with the previous findings of a study of the thalamic projection upon the telencephalon (Powell & Cowan, 1961), it appears that there is no direct relay of visual impulses from the thalamus to the telencephalon. In addition, a re-examination of the same material has failed to give any evidence of a projection to the telencephalon from the mesencephalic nuclei which receive retinal afferents. In view of these findings the identification of the pathways mediating the visual responses in the cerebral hemisphere found by Bremer *et al.* (1939) must await further investigation. Two possibilities may be mentioned. In the first place, as we have discussed previously, absence of retrogade cell degeneration following removal of the cerebral hemisphere does not necessarily exclude a direct projection from one of these nuclei. Secondly, the projection from one of these visual relay nuclei to the telencephalon could be through an indirect, multi-synaptic pathway; for example, fibres may pass from the tectum or relay nuclei to either the dorsal or central nuclear groups of the thalamus. From these nuclear groups impulses originating in the retina could then be relayed to the cortex or striatum. It may be noted here that Bremer *et al.* (1939) were unable to determine whether the responses which they obtained were cortical or subcortical in nature.

Although the visual centres of the avian brain, and particularly the tectum, are more highly developed than in the reptile there appears to be a basic similarity in the organization of the retinal projection in these two classes of vertebrates. After enucleation of the eye in *Lacerta*, Armstrong (1950) found terminal degeneration in the lateral geniculate nucleus, certain pretectal nuclei and the tectum. A further similarity is that none of these nuclei were found to undergo retrograde cell degeneration after removal of the telencephalon (Powell & Kruger, 1960), suggesting again that there is no direct relay to the telencephalon. The organization of the visual system in both birds and reptiles is therefore completely different from that found in the higher mammals where the neocortical projection from the thalamus has assumed a more prominent role than that to the midbrain.

Apart from the probable absence of a direct telencephalic relay system, the avian visual pathway is at least as complex as that found in the mammal. In all we have found eight distinct sites of termination of the optic nerve fibres. Following the classification of Huber & Crosby (1929) five of these nuclei are thalamic: the

nucleus lateralis anterior, the lateral geniculate nucleus, the nucleus synencephali, the ectomammillary nucleus and the nucleus externus. Of these nuclei, the lateral geniculate and ectomammillary nuclei have many features in common with the lateral geniculate nucleus and nucleus opticus tegmenti respectively of the reptilian brain. Thus, in the case of the lateral geniculate nucleus it is not only apparent that it has a similar topographical position, being adjacent to the optic tract in the ventrolateral part of the thalamus, but that in these two species these nuclei resemble one another in their morphology, both being readily divisible into an inner cellular and an outer plexiform layer. The ectomammillary nucleus of the avian thalamus and the nucleus opticus tegmenti of the reptile also closely resemble each other in their topographical position, and in receiving optic nerve fibres through the basal optic root: moreover, in both cases these fibres are larger and degenerate more rapidly than those of the main optic tract. In the absence of experimental evidence on the efferent connexions of these nuclei the validity of these homologies remains uncertain. The suggestion of Armstrong (1950) that the basal optic root and the nucleus opticus tegmenti of the reptile subserve visual reflex functions may apply equally well in the pigeon, especially in view of the observation of Huber & Crosby (1929) that the ecto-mammillary nucleus is connected with the oculomotor nuclei. It is difficult to suggest homologies for the nucleus lateralis anterior or the nucleus synencephali. It is possible, however, that the nucleus lateralis anterior is the avian homologue of the nucleus ovalis of the reptile to which earlier workers traced optic nerve fibres, although Armstrong (1950) found no terminal degeneration in this region. Similarly, it may be suggested, on topographical grounds, that the nucleus synencephali is homologous with the dorsal part of the lateral geniculate of reptiles. Although the nucleus externus is the smallest and least well defined of these thalamic nuclei in Nissl-stained material, the severity of degeneration in this nucleus after eye enucleation is particularly striking. Indeed, the density of boutons in the nucleus externus is as great as we have seen in any of the visual centres.

As is well known, the optic tectum is the principal site of termination of retinal fibres. Our degeneration experiments have confirmed the older observations based upon Golgi-stained material that the optic nerve fibres, after ramifying on the surface of the tectum, penetrate quite deeply into the stratum griseum et fibrosum superficiale (Cajal, 1911). Unequivocal terminal degeneration has been found throughout the outer half of this area reaching inwards as far as layer (*f*) of the classification given above which corresponds with the seventh layer of Cajal's description (cf. his figure 132). The fibres which penetrate most deeply have been found to undergo the earliest degenerative changes, while the maximum degeneration in the outer layers is not found until much later. This difference in the time course of degeneration in the superficial and deeper layers could be due either to different rates of degeneration in fibres ending at the various levels or to the fact that the collaterals given off to the more superficial layers by an incoming fibre fragment more slowly than the terminal portion of the axon. These experimental findings, although in agreement with the description of earlier authors using Golgi-stained material are at variance with the more recent work of Cragg, Evans & Hamlyn (1954) who, in their Golgi–Cox material, could not find terminal ramifications at levels deeper than the superficial plexiform layer (equivalent to our layers

(*a*) and (*b*)). In a later experimental study, however, Evans & Hamlyn (1956) illustrate terminal degeneration in what is clearly the 'radial fibre layer' of their classification, i.e. the deeper parts of the stratum griseum et fibrosum superficiale.

One feature of our findings on the tectum which was made most clearly in the Bodian preparations is the persistence of a substantial number of fibres in the deeper part of the stratum opticum even 26 days after removal of the contralateral eye. These fibres are more numerous in the ventro-medial part of the tectum where they can be traced forwards and medially into the optic chiasma. Their further course and direction of conduction is unknown, but there are three possible sites to which they may be directed or from which they may arise: the thalamus and hypothalamus of the same or opposite side, or the contralateral tectum. Cajal (1911) has described fibres entering the stratum opticum from deeper layers of the tectum but leaves their further course unspecified. In the tectum these fibres are only seen clearly in the Bodian preparations. The ease with which these fibres could be seen and traced in the Bodian preparations, especially after long periods, illustrates an additional advantage of this technique which might be used more widely in other studies.

Our findings on the nucleus isthmo-opticus present a number of problems for discussion. First, while there is unequivocal evidence of fibre degeneration in the isthmo-optic tract no terminal degeneration has been found in the nucleus after any survival period. The normal fibre plexus in the nucleus has always been clearly impregnated, but careful examination of material stained with all techniques has failed to show boutons or preterminal degeneration; it was not until 28 days after eye enucleation that some fibre break-up was seen, and by this time the cells of the nucleus showed fairly advanced chromatolysis. Secondly, this nucleus is the only site in the visual projection pathway in which definite cellular changes have been observed. These changes are first seen 18 days after enucleation and proceed over the following 6 weeks to severe cell-loss. The time course of the early cellular changes in this nucleus parallel those in the cranial nerve nuclei, but the later changes differ in that the isthmo-optic nucleus shows cell-loss whereas the cells of the cranial nerve nuclei progressively recover. A third unexpected feature of degeneration in this system is the finding that the initial signs of axonal degeneration in the isthmo-optic tract occur in that portion of the tract nearest to the nucleus and after longer survival periods appear to proceed centrifugally towards the optic chiasma. Finally, mention should also be made of the difference in time of appearance of degeneration in this tract according to the staining technique used; for example, although degeneration is clearly seen in the tract as early as 12 days in Nauta preparations it is not until 26 days after enucleation that the Bodian technique gives evidence of fibre degeneration. These findings provide further evidence that the silver techniques used in the present study stain different components of the fibre (cf. Evans & Hamlyn, 1956). Before dealing with the interpretation of these findings it is necessary to discuss the incidental findings on the IVth nerve which have a bearing on these problems. With the exception of the degree of the late cellular degeneration, the changes in the IVth nerve and its nucleus parallel, in every respect, the changes in the isthmo-optic pathway (including the different appearances with the various silver techniques). As there can be no doubt that the changes in this cranial nerve are retrograde in character there is a strong *a priori* case for the hypothesis that the

isthmo-optic system forms a centrifugal pathway. This hypothesis provides the simplest explanation for the absence of terminal degeneration in the isthmo-optic nucleus and of the severe cellular degeneration in the nucleus. It was first put forward by Perlia as early as 1889 to explain the complete atrophy of the isthmo-optic nucleus after experimental eye enucleation in the chicken. His finding has been confirmed by Jelgersma (1896) and by Huber & Crosby (1929). Taken by itself the finding of cellular degeneration in the nucleus is, of course, inconclusive and might be regarded as being transneuronal in character. As little is known about transneuronal degeneration in the avian brain this possibility cannot definitely be excluded, but from what is known of this process in the mammalian visual system it seems unlikely that it would have proceeded to such severe cell-loss as early as 2 months after enucleation in an adult animal (see Matthews, Cowan & Powell, 1960). The most conclusive evidence in favour of the hypothesis that the isthmo-optic pathway is a centrifugal one is provided by the work of Wallenberg (1898). Following lesions in the region of the isthmo-optic nucleus this author traced Marchi degeneration in the isthmo-optic tract, through the optic chiasma and nerve into the ganglion cell layer of the retina. In view of the considerable interest in centrifugal pathways at this time (see Granit, 1955; Livingston, 1959; Brindley, 1960) it is of some importance that Wallenberg's experiments be repeated using the more refined silver degeneration techniques.

The interpretation of the isthmo-optic system as a centrifugal pathway taken together with our observations on the IVth nerve suggests that retrograde fibre degeneration does not necessarily spread sequentially from the site of section of a nerve centripetally towards the cell body. Although there is a considerable amount of evidence on the sequence of changes close to the site of section of a nerve (see Cajal, 1928; Young, 1942; Guth, 1956), and while it is known that in some cases this degeneration may spread as far back as the cell body, it does not appear to have been recognized that degeneration may also commence close to the cell body and then spread centrifugally towards the site of section. It is difficult to assess the extent to which the slight traction exerted upon the optic nerve and extra-ocular muscles during enucleation may have contributed to this finding. It is well known that avulsion of a nerve may result in more severe degeneration than simple section, but it may be pointed out that in our experiments the nerves in the orbit were cleanly sectioned and nothing comparable to avulsion occurred.

Our material provides no evidence for an ipsilateral projection of the retina to either the diencephalon or the tectum. This is in agreement with the findings of nearly all previous workers (cf. Huber & Crosby, 1929; Evans & Hamlyn, 1956), but Polyak (1957) has described two small bundles which leave the contralateral optic tract in the pigeon, recross the midline in the suprachiasmal region and enter the ipsilateral 'rotund core' and the lateral geniculate nucleus. Despite repeated examination we have never observed degeneration in the nucleus rotundus (? 'rotund core'), and as it is difficult to assess the significance of the few boutons seen in the ipsilateral lateral geniculate nucleus (comparable numbers being seen in normal material) we are unable to confirm Polyak's findings. Nor have we found any distinct bundle recrossing the midline in the manner he describes or passing into the ipsilateral optic tract as Armstrong (1950) has found in the lizard.

SUMMARY

1. The central projection of the retina has been studied in the pigeon using a variety of silver degeneration techniques.

2. Within 5 days of eye-enucleation the optic nerve is severely degenerated and the degenerating fibres appear to decussate completely in the optic chiasma to enter the ventral part of the contralateral optic tract. From the optic tract this degeneration can be traced into the following thalamic nuclei: nucleus lateralis anterior, the lateral geniculate nucleus and the nuclei externus and superficialis synencephali. More posteriorly fibres pass from the marginal optic tract to the outer layers of the optic tectum and to the tectal grey. Coarse degenerating fibres leave the dorsum of the optic chiasma to form the basal optic root; this can be followed along the ventral aspect of the diencephalon to its termination in the ectomammillary nucleus.

3. Severe cellular degeneration in the isthmo-optic nucleus and the unusual nature and time course of the fibre degeneration in the axillary or isthmo-optic tract are suggestive of retrograde degeneration in a centrifugal system, which is in agreement with the findings of earlier authors.

4. An appreciable number of normal fibres persist in the stratum opticum of the tectum and can be followed through the marginal optic tract to the region of the optic chiasma; their precise origin and termination are, however, unknown.

5. No degeneration has been found in the hypothalamus or in those thalamic nuclei which are known to project upon the telencephalon.

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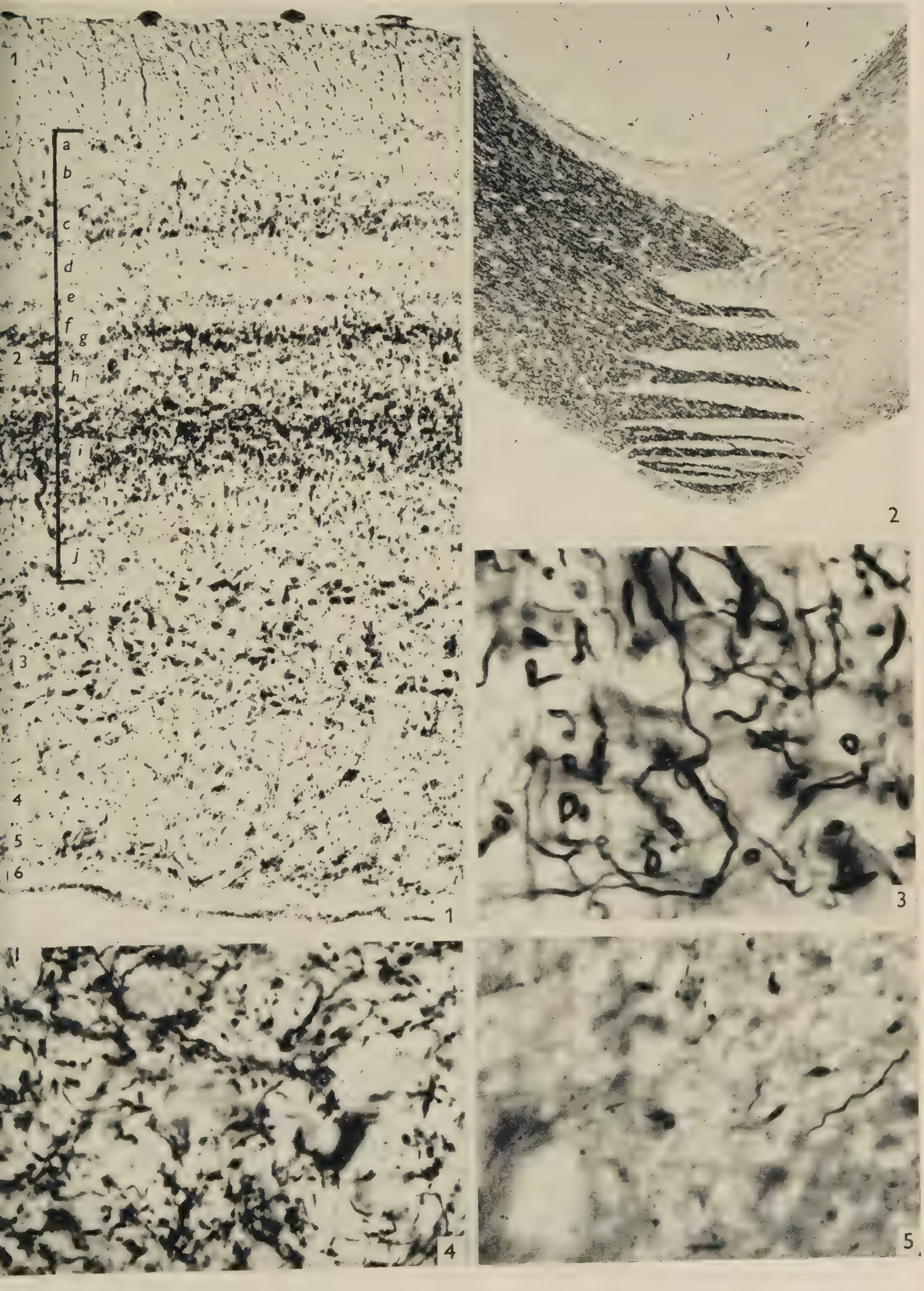
ABBREVIATIONS

BOR	Basal optic root	*NLA*	Nucleus lateralis anterior
EMN	Ectomammillary nucleus	*NO*	Nucleus ovoidalis
EN	Entopeduncular nucleus	*NR*	Nucleus rotundus
IOT	Isthmo-optic tract	*NSM*	Nucleus superficialis synencephali
LFB	Lateral forebrain bundle	*NSR*	Nucleus subrotundus
LGN	Lateral geniculate nucleus	*NSP*	Nucleus superficialis parvocellularis
LHN	Lateral habenular nucleus	*NSPI*	Nucleus spiriformis
MLB	Medial longitudinal bundle	*OC*	Optic chiasma
MHN	Medial habenular nucleus	*ON*	Optic nerve
MOT	Marginal optic tract	*OT*	Optic tract
NDL	Nucleus dorsolateralis	*PC*	Posterior commissure
NDM	Nucleus dorsomedialis	*SPN*	Subpretectal nucleus
NE	Nucleus externus	*TG*	Tectal grey
NIO	Nucleus isthmo-opticus	IV	IVth nerve nucleus



COWAN, ADAMSON AND POWELL—AN EXPERIMENTAL STUDY OF THE AVIAN VISUAL SYSTEM

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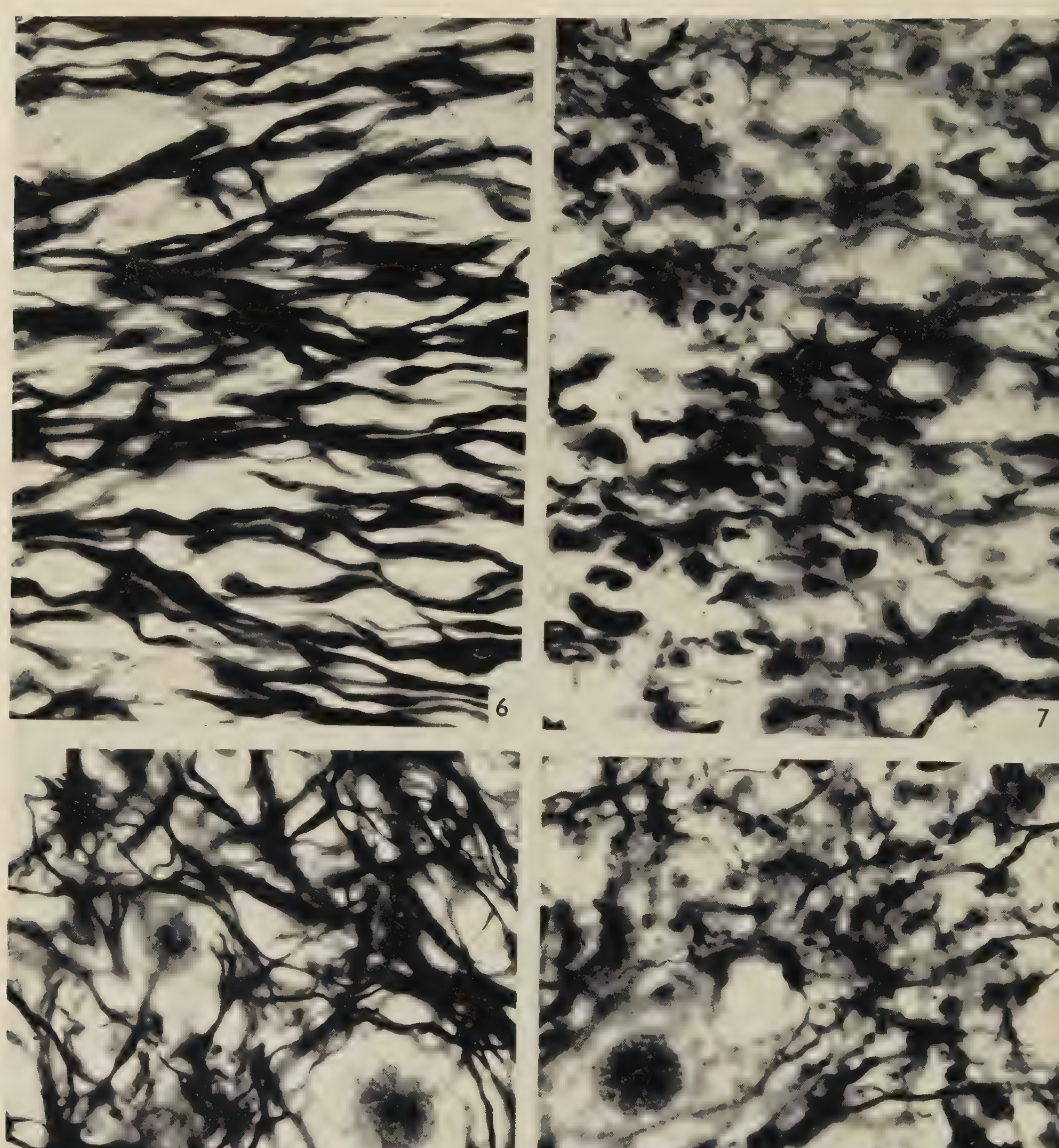

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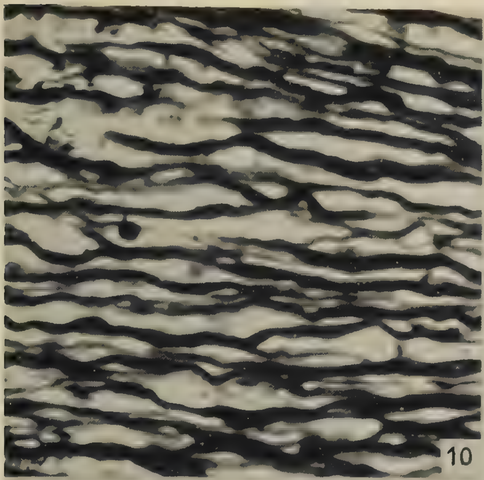

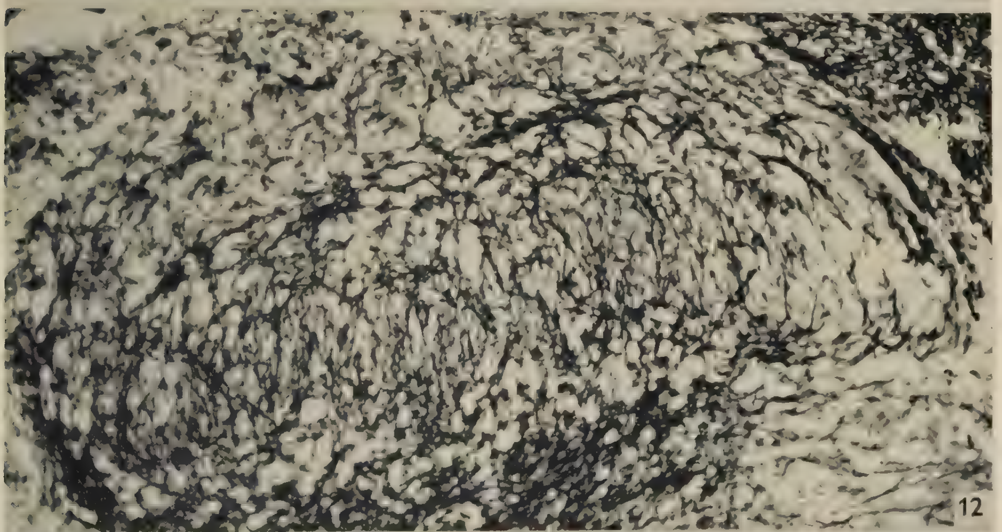

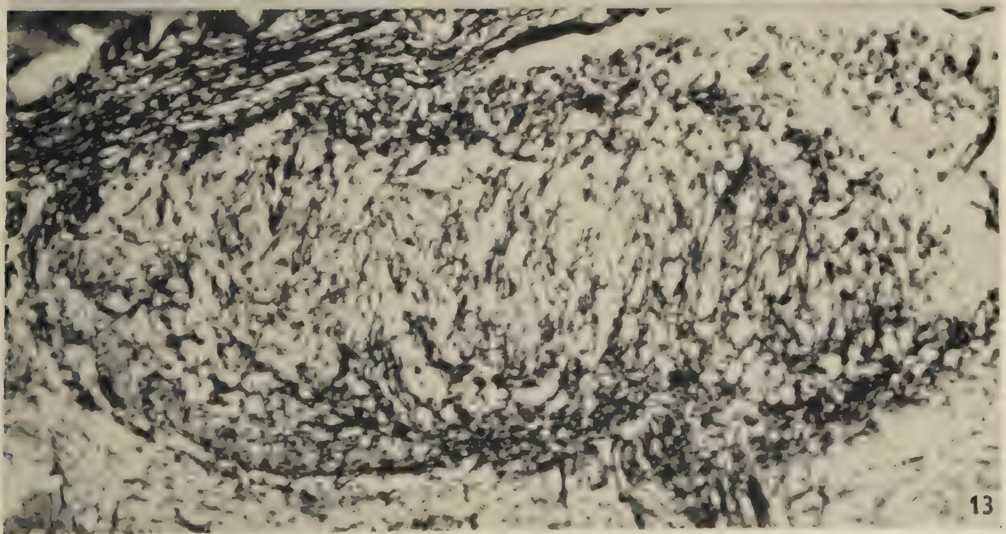

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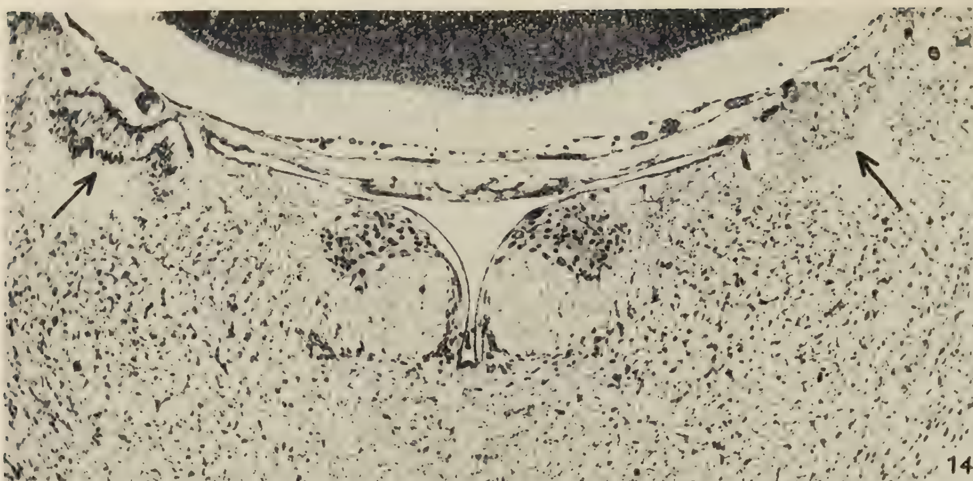
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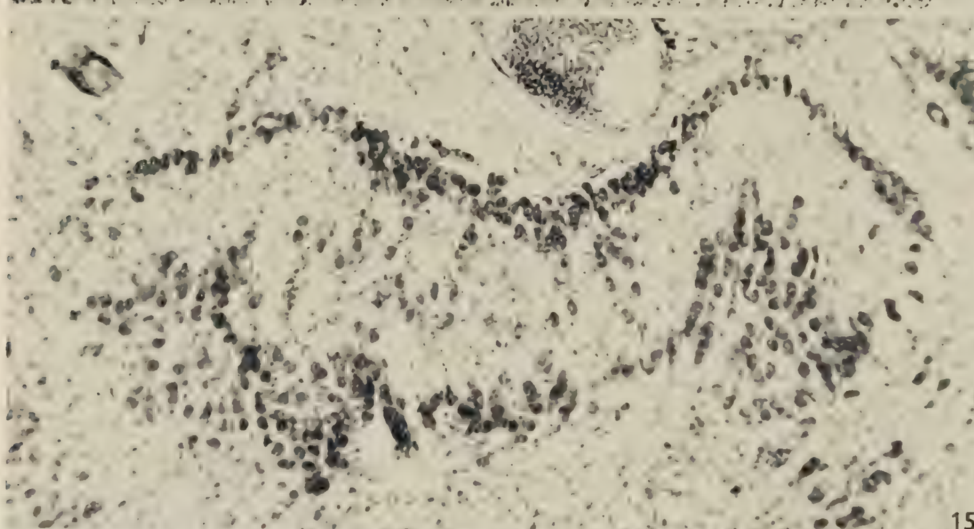
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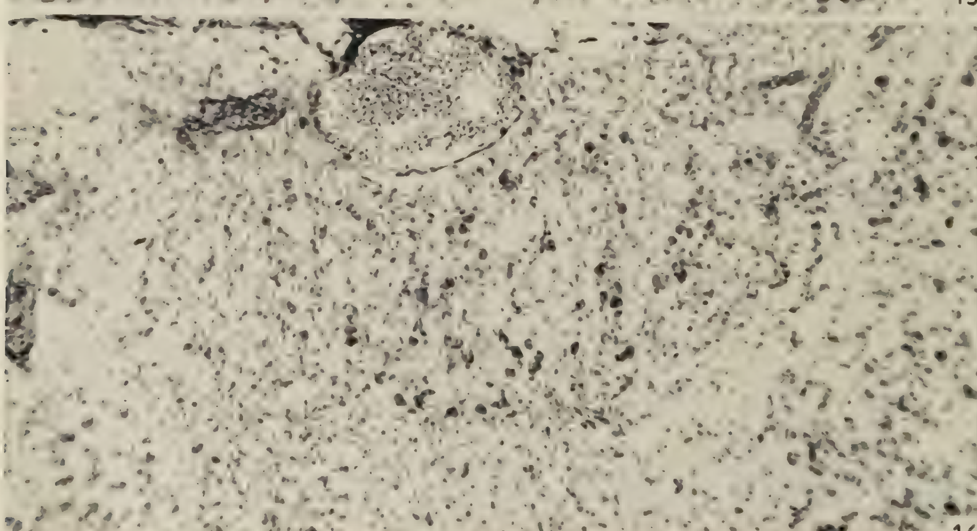
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COWAN, ADAMSON AND POWELL—AN EXPERIMENTAL STUDY OF THE AVIAN VISUAL SYSTEM

EXPLANATION OF PLATES

Plate 1

Fig. 1. Section through the optic tectum of a normal pigeon to show the lamination described in the text. Thionine preparation. ×96.

Fig. 2. The optic chiasma 26 days after eye-enucleation as seen in coronal section in a paraffin Nauta preparation. ×32.

Fig. 3. Boutons in layers (*d*) of the stratum griseum et fibrosum superficiale of the tectum 12 days after enucleation of the contralateral eye. Bodian method. ×1720.

Figs. 4, 5. Nauta degeneration in the nucleus externus (fig. 4) with control from the opposite side (fig. 5). Expt. AP1 (18-day survival). ×630.

Plate 2

Figs. 6, 7. Fibres from the normal basal optic root (fig. 6) and from the side contralateral to the eye-enucleation (fig. 7). AP3A (12-day survival). Glees and Marsland technique. ×1150.

Figs. 8, 9. The normal fibre plexus in the ectomammillary nucleus (as seen in the Glees sections) (fig. 8), and 12 days after eye-enucleation (fig. 9). ×1150.

Plate 3

Figs. 10, 11. Fibres in the normal trochlear nerve (fig. 10) and on the affected side 26 days after section (fig. 11). Paraffin Nauta preparation. ×1150.

Figs. 12, 13. The normal fibre plexus of the isthmo-optic nucleus (fig. 12), and 26 days after eye-enucleation (fig. 13) to show loss of fibres in the central part of the nucleus. ×154.

Plate 4

Fig. 14. Low-power photomicrograph through the midbrain at the level of the IVth nerve nucleus to show the position of the normal isthmo-optic nucleus (indicated by arrow on left) and of the degenerated nucleus on the right. Expt. EP2 (thionine preparation). ×22.

Figs. 15, 16. Photomicrograph of the normal (fig. 15) and degenerated isthmo-optic nucleus (fig. 16) from experiment EP2 at a higher magnification to show the severity of the cellular degeneration 2 months after eye-enucleation. ×140.

THE REACTION OF THE EAR CARTILAGE OF THE RABBIT AND GUINEA-PIG TO TRAUMA

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INTRODUCTION

In the course of investigations into the behaviour of autografts of ileum and the lining of the urinary bladder transplanted to the ear in rabbits (Joseph, 1960), it was noticed that in many animals the cartilage of the ear proliferated and formed bone. It was difficult, however, to decide whether the bone formation was due to the presence of the grafts or to local tissue injury alone, since osseous tissue developed in some control animals. Proliferation of the cartilage was observed after its removal by Schwalbe (1878) and Matzuoka (1904) but they did not report the formation of bone. Asami & Dock (1920) found that transplantation of ear cartilage to a subcutaneous site in rabbits resulted in bone formation, an observation which Mauclaire (1920) pointed out had already been made in 1898. Clark & Clark (1942) noticed that proliferation of cartilage occurred at the edges and/or in the middle of some of their ear chambers with the occasional formation of bone after comparatively long periods of time (2 months for cartilage at the edge and 4 months at the centre). In the present investigation an attempt was made to obtain more details of the histological changes which occur following different degrees of trauma to the cartilage and the incidence of bone formation. Since the cartilage reaction in the rabbit's ear might be species specific, similar experiments were carried out on guinea-pigs.

MATERIAL AND METHODS

Adult rabbits weighing 2–3 kg. and guinea-pigs weighing 0·4–0·5 kg. of mixed stock were used. In the rabbits two main types of operation were carried out under Nembutal anaesthesia supplemented by ethyl chloride as required. The first consisted of the removal of one or two areas of skin 1×1 cm. from the inside of the ear followed by the removal of $0{\cdot}5 \times 0{\cdot}5$ cm. of the central part of the exposed cartilage. The wound was dressed with penicillin tulle gras, surgical gauze and plaster of Paris. The dressings were removed after either 5 or 10 days and the wound was allowed to heal without further dressings. In the other operation the whole thickness of the ear $2{\cdot}5 \times 2{\cdot}5$ cm. in area was removed. The bleeding from the edges was stopped by pressure and the edges were allowed to heal without dressings.

Subsequently in the first group, the whole thickness of the ear containing the healed area was removed, and in the second group 3–4 mm. of the edges of the hole outside the original cut edge were removed. The specimens were divided into four by transverse and longitudinal cuts and fixed in 80 % alcohol, Bouin's fluid, formaldehyde and Helly's fluid and three or four specimens after 5, 10, 20, 30, 60, 100, 120, 150, 180, 200 and 230 days were obtained. Sections were cut at $7\,\mu$ and stained with haematoxylin and eosin. Some sections were stained with thionin to demonstrate

metachromasia, some with Verhoeff's stain for elastic fibres and some with Van Gieson's stain for collagen. In order to investigate the effect of different types of trauma three further operations were performed. In three ears a much narrower piece of cartilage was removed consisting of a triangular piece of the whole thickness of the ear, 1·5 cm. long and 2–3 mm. at its base. Secondly, a slit 1·5 cm. long was made in the whole thickness of the ear and thirdly a small circular area of skin 2 mm. in diameter was removed and the underlying surface of the cartilage scraped. All these were allowed to heal without dressings. These were left for 60 days and the whole thickness of the ear containing the healed area was removed and fixed in Bouin.

In the guinea-pigs, under Nembutal anaesthesia, an area of skin 1×1 cm. was removed from the inside of one ear and the central part of the exposed cartilage, measuring $0{\cdot}5 \times 0{\cdot}5$ cm., was removed. A wound 1×1 cm. was made through the whole thickness of the other ear. Both ears were left without dressings and allowed to heal. Five specimens after 25 days, six after 60 days and nine after 100 days were obtained. Each specimen was fixed in Bouin's fixative and sections were cut at 7μ and stained with haematoxylin and eosin.

RESULTS

Rabbits

The detailed histological changes were followed in those animals in which a considerable amount of cartilage had been removed. Five days after wounding, granulation tissue was seen near the edges of the cut cartilage which itself showed degenerative changes for a short distance from the cut (Pl. 1, fig. 1). These were indicated by a loss of basophilia from the ground substance together with a loss of metachromasia usually extending further than the loss of basophilia. Vacuolation of the chondrocytes occurred near the cut edge. Both the fibrous and the chondrogenic layers of the perichondrium showed proliferative changes, together with migration of new cells. Sometimes the growing perichondrium ensheathed the cut edge of the cartilage, apparently 'sealing it off'. More often other perichondrial cells, mainly those of the fibrous layer, migrated into the granulation tissue adjacent to the cut edge. The cells in the superficial layers of the cartilage were somewhat larger than normal and more rounded. These changes occurred on either or both surfaces of the cartilage plate.

After 10 days the cartilage showed similar but more extensive changes than those seen in the 5-day specimens. The cut end of the cartilage was completely sealed by granulation tissue and growth and migration of perichondrial cells, most of which appeared to be derived from the fibrous layer of the perichondrium. This increase in cellularity was probably the beginning of new cartilage formation. In some specimens the two surfaces of the cartilage were different in that there was loss of cellularity on only one side (Pl. 1, fig. 2). In those animals in which the skin was removed this change frequently occurred on the side opposite to that from which the skin had been removed. A loss of basophilia and metachromasia of the ground substance were associated with the loss of cellularity.

After 20 days the cartilage showed a considerable proliferation of the chondrogenic layer. Sometimes there was also a proliferation of young chondrocytes (Pl. 1, fig. 3)

which was due to mitosis (Pl. 1, fig. 4). The increase in cartilage was frequently confined to only one surface and was greatest nearer the cut edge. Where proliferation was considerable, the new cartilage was very similar to 'callus cartilage', that is the cartilage cells were separated from each other by considerable amounts of ground substance (Pl. 1, fig. 5) in which were embedded large numbers of fine collagen fibres. There was no evidence of proliferation of the mature chondrocytes of the old cartilage. Near the cut edge the cartilage showed the beginning of calcification (Pl. 1, fig. 6). In some of those animals in which the skin of only one side had been removed the new cartilage surrounded the cut end of the old cartilage, and gave the appearance of the beginning of bridging the gap caused by the removal of the piece of cartilage (Pl. 2, fig. 7). The new ground substance of the cartilage showed more metachromasia than the old (Pl. 1, fig. 5).

After 30 days the main changes seen were an increase in the amount of new cartilage and a maturing of the cartilage which had developed earlier. Elastic fibres were seen for the first time in the ground substance of the new cartilage which was still markedly metachromatic.

After 40 days there was an increase in the amount of new cartilage but metachromasia was reduced as compared with that of 30 days. In some specimens the beginning of bone formation was seen with the breakdown of primary areolae in the oldest part of the new cartilage and the formation of secondary areolae. Calcification of the cartilage was also seen particularly in these areas (Pl. 2, fig. 8).

From 40 days onwards and up to 200 days, bone was identified in all specimens in which cartilage had been removed. The amount of bone apparently increased up to 100 days and then remained more or less stationary. The bone itself developed cavities containing bone-marrow cells (Pl. 2, fig. 9). The proliferation of cartilage appeared to stop at about 60 days after which it became a mixture of fibro- and elastic cartilage with the chondrocytes lying in large lacunae as in normal cartilage.

In the remaining three groups of rabbits, after 60 days all ten ears showed proliferation of cartilage. There was no bone in any of three ears in which a slit had been made; two out of the three ears in which a triangular area had been removed showed bone and bone was seen in two out of four in which the surface of the cartilage had been scraped. It should be added that in the ears with a slit cut through the whole thickness, the cartilage had healed across the gap produced by the operation and that the ear in which a triangular area had been removed and bone had not developed had also healed across the gap. There were necrosis and loss of cartilage in the two ears in which the cartilage had been scraped and bone had developed. The other two specimens without bone showed practically no loss of cartilage.

Guinea-pigs

After 25 days only one out of five ears showed proliferation of cartilage, but in all fifteen ears after 60 or 100 days proliferation was seen (Pl. 2, fig. 10). This growth of cartilage was variable in extent but in all specimens appeared to be completed, that is one did not get the impression that after 60 days the process of proliferation was continuing. In some specimens the new cartilage, although different in structure, appeared to be replacing that which had been removed, and contained elastic fibres (Pl. 2, fig. 11); it did not spread extensively and haphazardly into the surrounding

tissue (Pl. 2, fig. 10) as was frequently seen in the rabbits' ears. Bone did not develop in any of the guinea-pigs' ears.

DISCUSSION

These investigations have shown that if cartilage is removed from, or necroses in, the ears of rabbits or guinea-pigs the remaining cartilage proliferates and in many cases attempts to repair the gap. In addition, in rabbits this proliferation is usually extensive, spreads into the adjacent tissue, and after 40 days invariably results in the formation of bone. The findings suggest that in the rabbit bone is not formed unless there is an actual loss of cartilage. In comparison the process of regeneration is very much slower in hyaline (costal) cartilage in which bone does not develop (Rao, 1954). The formation of bone in the ears of the rabbits may be regarded as a somewhat curious development since as was pointed out by Clark (1958) it is the hyaline cartilage of the larynx and ribs which calcifies and/or ossifies with age whereas elastic cartilage rarely if ever shows these changes. Previous observers including Schwalbe (1878) and Matzuoka (1904) observed the proliferation of the cartilage of the ear if some of it was removed or lost, but did not notice that bone appeared. Clark & Clark (1942) commented on the appearance of cartilage and bone in their ear chambers but in their experiments bone appeared infrequently and subsequently regressed.

The absence of the development of bone in the ears of guinea-pigs suggests that there is a species difference with regard to the response of the elastic cartilage of the ear to trauma. There is, however, a quantitative difference between the amount of cartilage which develops in the ear of the rabbit and the guinea-pig in this experimental situation, and the smaller amount of proliferation of cartilage in the guinea-pigs' ears may account for the absence of bone. One cannot suggest why the proliferation of cartilage is much greater in the rabbit's ear than in that of the guinea-pig. In general these investigations indicate that tissues, apparently the same, react in a different way in different animals to what is almost the same traumatic procedure.

The new cartilage appears to be produced by the chondrogenic layer of the perichondrium left behind after the operation, and the cells may respond to chemical stimuli due to trauma. It is also possible that the loss of continuity of the tissue resulting from its removal acts as a stimulus. Although in all the animals studied the wound is covered after 15–20 days, it is obvious that deep to the new epidermis further changes go on for some time so that the changes initiated by trauma continue long after the external tissues have apparently healed.

As a cautionary note one may add that in these experiments bone is frequently seen separated from the underlying normal cartilage. This observation suggests that the ear of the rabbit is an unsuitable site for transplantation experiments in which bony changes are being investigated, since it is very easy to injure the cartilage when removing or separating the skin of the rabbit's ear from the underlying tissue (Chytilová, Kulhánek & Horn, 1959). Similarly, care is needed in interpreting the results obtained in the ears of rabbits following injections which produce proliferation of cartilage and 'metaplastic bone transformation' (Menkin, 1960).

SUMMARY

1. The cartilage of the ears of rabbits and guinea-pigs was removed or injured. The remaining cartilage was examined after various intervals of time.

2. In both rabbits and guinea-pigs, loss of cartilage was followed by proliferation of the remaining cartilage. Bone was found in the ears of all rabbits after 40 days but not in the ears of the guinea-pigs even after 100 days.

3. The detailed histological changes of these processes are described.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Cut end of cartilage after 5 days showing degenerative changes at edge of cartilage and proliferation of perichondrial cells (rabbit, H. & E.).

Fig. 2. Cartilage after 10 days showing formation of granulation tissue round cut edge and loss of cellularity on one side (rabbit, H. & E.).

Fig. 3. Cartilage after 20 days showing proliferation of young chondrocytes (rabbit, H. & E.).

Fig. 4. High-power view of fig. 3 to show two nuclei in metaphase (arrowed).

Fig. 5. New cartilage after 20 days showing large amount of ground substance (rabbit, H. & E.).

Fig. 6. New cartilage after 20 days showing early calcification indicated by darker areas in upper half of picture (rabbit, alizarin).

Plate 2

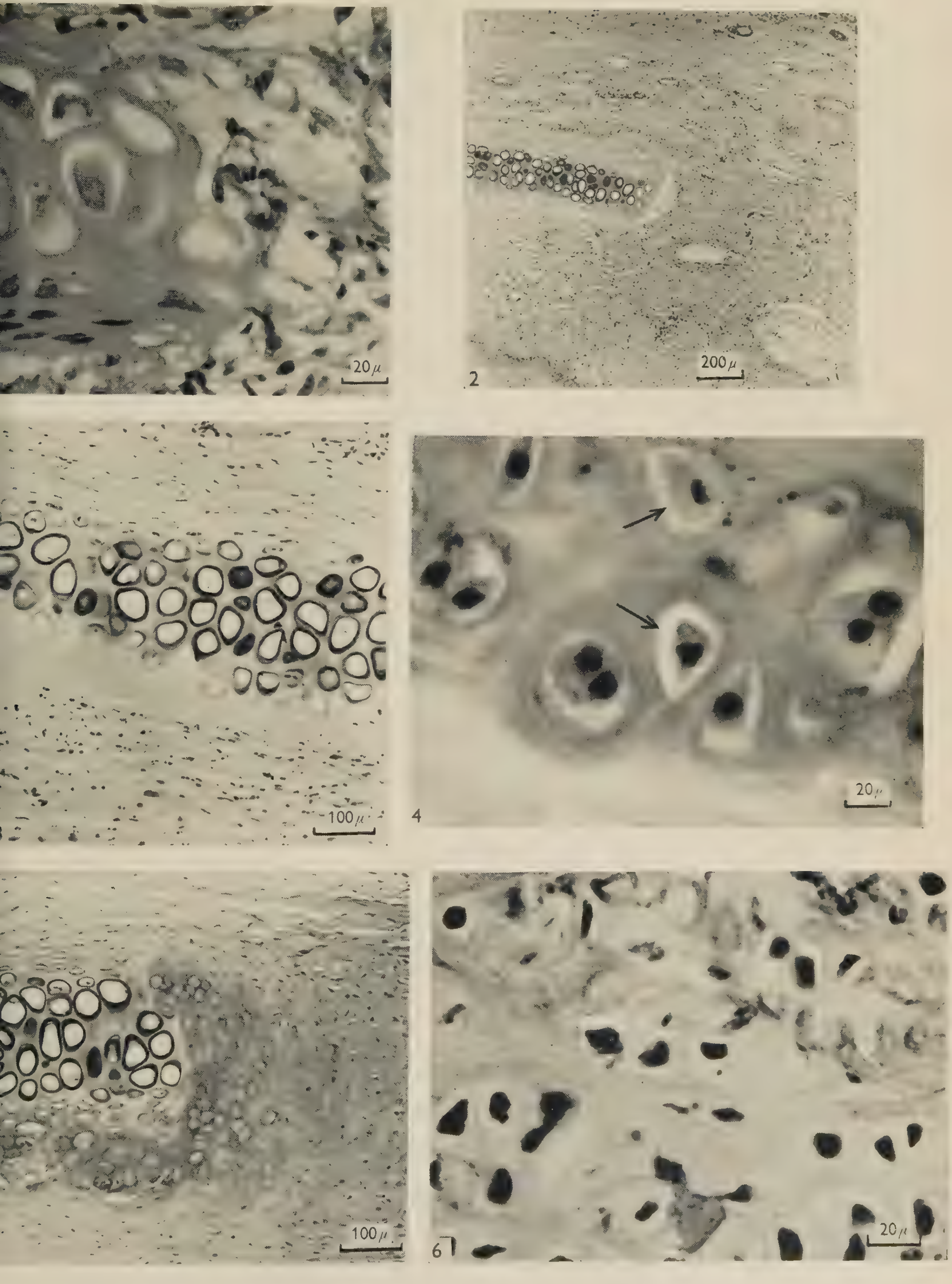
Fig. 7. New cartilage after 20 days showing growth which is bridging the gap produced by original removal (rabbit, H. & E.).

Fig. 8. New cartilage after 40 days showing areas of calcification (rabbit, H. & E.).

Fig. 9. Bone developing in new cartilage after 60 days (rabbit, H. & E.).

Fig. 10. Proliferation of cartilage after 60 days (guinea-pig, H. & E.).

Fig. 11. Appearance of elastic fibres in new cartilage after 60 days (guinea-pig, Verhoeff's stain).



JOSEPH, THOMAS AND TYNEN—EAR CARTILAGE OF RABBIT AND GUINEA-PIG

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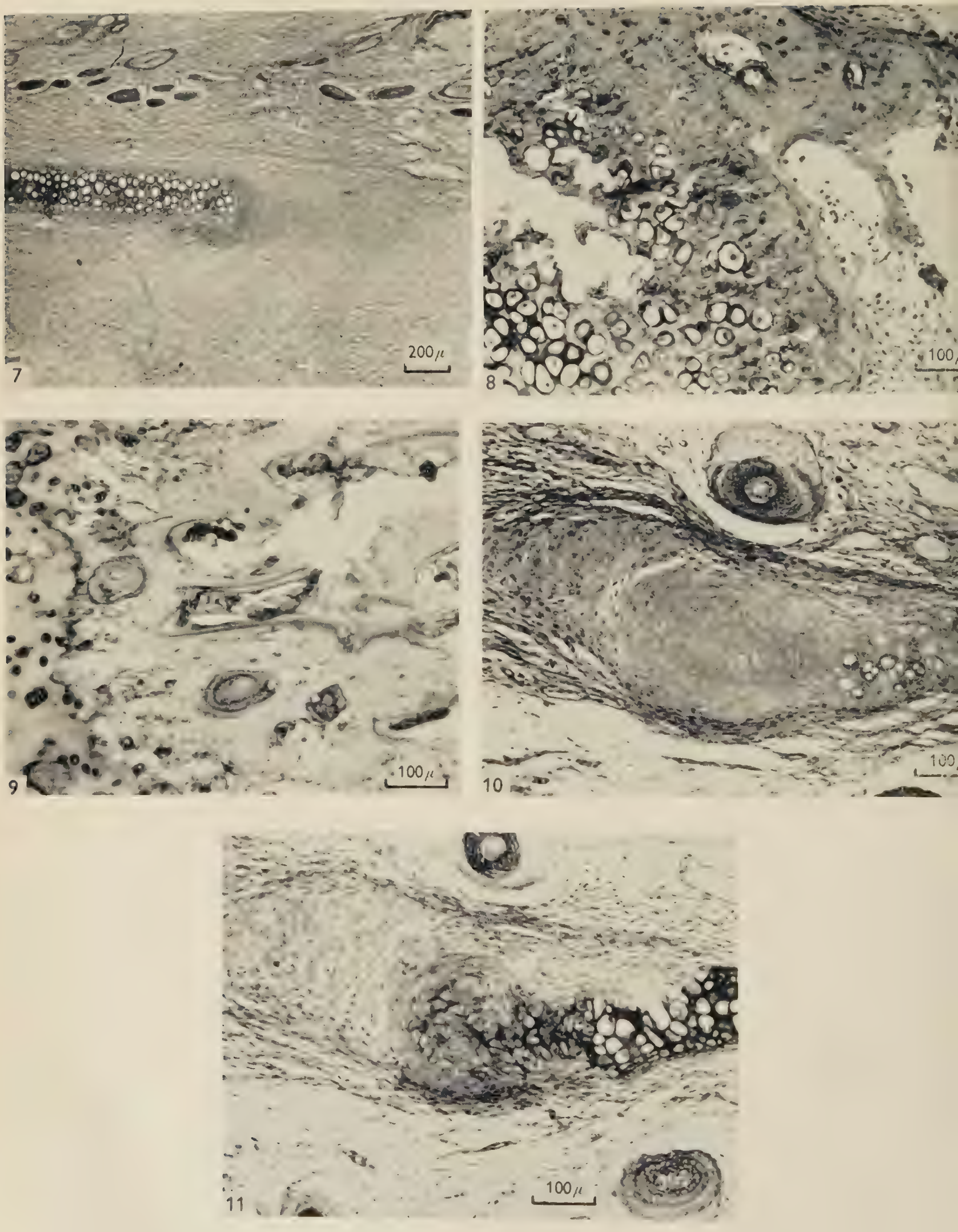

JOSEPH, THOMAS AND TYNEN—EAR CARTILAGE OF RABBIT AND GUINEA-PIG

THE HISTOLOGY AND HISTOCHEMISTRY OF THE DERMIS IN HAIRY AND NON-HAIRY PARTS OF THE HUMAN SKIN WITH SPECIAL REFERENCE TO BALDNESS

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Remarkably little in the way of substantial scientific fact is known about baldness in the adult male. It is too common to be regarded as an outright pathological state and yet it cannot be lightly accepted as a physiological phenomenon. Heredity is acknowledged as playing a major part in its appearance but experimental work with hormones has given conflicting and unpredictable results.

The normal cycle of hair growth has been established for man and animals and its variations after gonadectomy, adrenalectomy and hypophysectomy have been recorded in the male and female. Parnell (1949) showed that in the rat the hair growth cycle was only part of a more extensive series of changes within the skin involving even the sebaceous glands; there is, in fact, a 'skin cycle' and, according to Rothman (1954), in the early 'active' stage of the cycle in the mouse the epidermis and adipose layer of the skin become two or three times thicker and the dermis increases by 50 %. Histochemical changes in the basophilic material can be detected in the hair follicle and external root sheath during the cycle.

The present study, chiefly histological and histochemical, has been restricted to (*a*) the ground substance of the papillary layer and (*b*) the basement membrane in human skin from both sexes at different ages and in hair-bearing and non-hair-bearing parts of the body.

MATERIALS AND METHODS

Two hundred and nine pieces of human skin, hair-bearing and non-hair-bearing, were collected from eighty subjects of both sexes from foetal life to 90 years of age. Most of the material was fixed within 8 hr. but some specimens were not obtained until more than 36 hr. after death. All specimens were fixed in 10 % formalin for 72 hr., dehydrated in four changes of dioxan, embedded in wax (m.p. 58° C.) and sectioned at 10 μ. Thicker sections were preferred for the various destructive methods involved in digestion of the tissues (see below).

To demonstrate metachromasia 0·1 and 0·05 % aqueous and alcoholic solutions of toluidine blue (G. T. Gurr, batch no. 1040) were used after they had ripened for a few days. With ordinary techniques, however, the metachromasia seen in the sections immediately after staining disappeared during dehydration —a false metachromasia. Similar results were obtained with azure A. Variations in technique as

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suggested by Pearse (1953) and others were of no value in retaining the metachromasia.

Mucopolysaccharides of the metachromatic type, for example, hyaluronic acid and chondroitin sulphuric acid are present in approximately equal concentrations in the skin (Pearse & Watson, 1949), but Sylven (1954) showed that concentrations of hyaluronic acid less than 2% w/vol. gave no stable metachromasia. The strong chondroitin sulphuric acid—protein complex (Rothman, 1954) was suspected of being responsible for the lack of a stable metachromasia and accordingly attempts were made to free the mucopolysaccharides with pepsin, 2 mg. (Difco, batch no. 8308, 1:3000) in each ml. of N/50 HCl at pH 1·8 (Pearse, 1960). Incubation in this solution at 37° C. for 45 min., staining in 0·1% aqueous toluidine blue for 10 min., dehydration and clearing in xylol gave faint but definite metachromasia. Control sections were incubated in the acid solution only.

Unfortunately the number of specimens surviving the digestion was very small and in order to retain the sections on the slide not only for the 45 min. but for a much longer period, the technique of Goetsch, Reynolds & Bunting (1952) for the identification of enzymes in paraffin sections was employed, viz. omission of xylol treatment prior to hydration. In this way, pepsin digestion could be prolonged for several hours but, of course, the presence of the paraffin itself made longer treatment necessary. Although loss of sections was still a hazard, the technique was maintained and a standard method devised as follows.

Digestion of sections in N/50 HCl pepsin solution was continued for 16 hr.: controls were incubated in N/50 HCl only. A parallel series of pepsin-digested material was washed in distilled water and further incubated in Hyalase solution (Testicular hyaluronidase, ovine 1500 international units in 100 ml. of 0·85 normal saline) for 4 hr. at 37° C. All three series were stained together in 0·1% aqueous toluidine blue for 15 min. at pH 4·5, rinsed in distilled water, dehydrated in alcohol and xylol and mounted in DPX.

The ground substances and basement membrane were studied after treatment by the PAS technique using 'Cold Schiff' reagent (Lillie, 1954).

Sections, after passing through xylol and absolute alcohol, were collodionized and then hydrated, treated with 0·5% periodic acid for 10 min., washed in running water for 5 min., placed in Schiff reagent for 10 min., left in 2% sodium bisulphite solution for 2 min. and again washed in running water for 10 min. Controls were treated in the same way except for the omission of the periodic acid bath. Sections were then treated with 95% alcohol, alcohol-ether, absolute alcohol, and mounted in DPX. A few sections were subjected to acetylation by incubation at room temperature for 8 hr. in 16 ml. of acetic anhydride and 24 ml. of anhydrous pyridine.

For the further identification of collagen and the pattern of the collagen bundles in the dermis, a series of sections was treated with trichrome stain (Masson, 1928).

RESULTS

Following pepsin digestion in the paraffin ribbon and toluidine blue staining as described above, stable or alcohol fast metachromasia could be seen in the papillary (subepidermal) layer of the dermis in all the typically hairy parts of the skin, for

example, the hairy scalp, forehead, beard area, chest, abdomen and pubic region (Pl. 1, fig. 1). In each case the metachromatic reaction was absent after using hyaluronidase. Bald areas of the scalp (Pl. 1, fig. 2), compared with other regions of the same individual (Pl. 1, fig. 3), showed little or no metachromasia in the papillary layer. These findings were characteristic of material fixed within 8–12 hr. of death. Skin which had not been obtained and fixed until more than 36 hr. after death was strongly metachromatic throughout the papillary layer whether it came from a bald or hair-bearing area; in fact, the reaction was more pronounced in the bald parts (Pl. 1, figs. 4, 5). Junctional areas of bald and hairy scalp showed appreciable metachromasia irrespective of the time of fixation, while the forehead could be ranked with hairy parts of the skin.

Definition of the papillary layer of the dermis in H and E and trichrome-stained material was possible because of the finer texture of the fibrillar material comprising that layer. Not only was the metachromasia confined to the papillary layer but it was noted also, with increase in age, and particularly in skin from bald parts, that the depth of the papillary layer and hence the extent of the metachromasia were reduced to about one-sixth or less of its initial thickness by the encroachment of coarse collagenous bundles from the reticular layer of the dermis. Trichrome staining was helpful in showing that even within the papillary layer there was a progressive thickening of fibres with age and particularly in the bald areas where the diameter of the fibres may be three or four times that in the foetal skin (Pl. 2, figs. 6, 7). The skin of the palm and sole, both foetal and adult, showed metachromasia throughout the papillary layer although the collagen fibrils in it were thicker than elsewhere.

The PAS reaction in the basement membrane of the skin is normally strongly positive but in the ground substance only moderately so (Pl. 2, figs. 8, 9). Stained in this way the basement membrane in skin from most parts of the body was thin and discontinuous, increasing in thickness (approximately three times) with age (Pl. 2, figs. 8, 9). In bald areas of the scalp (Pl. 2, fig. 10), this structure was enormously thickened (approximately six times) compared with any other region of the same subject. Even in the palm and sole it remained thin and poorly defined.

None of these results was affected by sex or by delay in fixation up to 12 hr.

DISCUSSION

The production of a stable or alcohol-fast metachromasia in the papillary layer of the dermis by aqueous toluidine blue after digestion of the specimen by pepsin confirms the view that the metachromatic elements are normally firmly combined with a protein or proteins although these results give no indication of the nature of the protein. It remains to identify these elements and to discuss their significance in hairy and bald parts of the skin. Pearce & Watson (1949) using Meyer & Chaffee's (1941) method for isolating mucopolysaccharides from pig skin, showed that hyaluronic acid and chondroitin sulphuric acid were present in the skin of amputated limbs of men and women in concentrations of $24{\cdot}5 \pm 5{\cdot}7$ and $26{\cdot}2 \pm 4{\cdot}7$ mg. per 100 g. of fresh skin, respectively. No metachromatic reaction is possible with hyaluronic acid in concentrations of less than 2% according to Sylven and 1% according to Meyer (1947). Thus we must assume that it is the sulphated mucopolysaccharides

alone which are responsible for the metachromasia found in the papillary layer of the dermis.

Meyer, Davidson, Linker & Hoffman (1956) have studied the types and quantities of acid mucopolysaccharides isolated from different tissues and fluids and claim that skin contains only two of these sulphated mucopolysaccharides, chrondoitin sulphates B and C and the latter only as a small and variable fraction. According to these authors chondroitin sulphate B is unaffected by testicular hyaluronidase while chondroitin sulphate C is destroyed by the enzyme. Pearse (1960), however, maintains that it is not possible to distinguish the sulphated mucopolysaccharides in tissue sections by the hyaluronidase reaction, pointing out that the metachromatic substances of skin resist neither the testicular nor the bacterial type of hyaluronidase.

It may not be quite certain from the present investigation that there is a progressive diminution with age of metachromasia in the papillary layer of the skin, but the absence or marked diminution of metachromasia in the bald scalp compared with other regions of the body suggests that there is a loss of the sulphated mucopolysaccharides where hair has ceased to grow. The return or reappearance of the metachromasia in material which had not been fixed until more than 36 hr. after death calls for reconsideration of this idea, because under these circumstances the metachromasia appears to be more intense in the bald areas than elsewhere in the same subject. Postmortem autolysis, according to Fruton & Simmonds (1953), is the result of proteolytic enzyme activity, three separate proteinases having been dentified. Of these, cathepsin A is identical in its specificity to pepsin. Where fixation of the skin had been delayed and in the slightly acid medium which develops in tissues after death, this cathepsin can apparently degrade the tissue protein to an appreciable extent before artificial pepsin digestion is begun. We must assume, then, that protein breakdown and therefore release of the chondroitin sulphate is more successful in the case of partially autolysed tissue than fresh tissue. Accordingly, instead of interpreting the results as showing a diminution of metachromatic elements in the papillary layer of the dermis we suggest an increased concentration marked by a firmer combination between the protein and mucopolysaccharides. This is in agreement with the view that, with age, there is a decrease in hyaluronic in the tissues and an increase in the sulphated mucopolysaccharides, baldness being an exaggeration of the normal ageing process.

Regarding the increased concentration of chondroitin sulphate in the papillary layer of the dermis under these circumstances, it is interesting to note that Hoffman, Linker & Meyer (1957) claim that 'Chondroitin sulphate B appears to be associated with the coarser type of collagen fibres'. This is substantiated by the thickness of the fibrillar material and its more irregular pattern in the papillary layer over the age of 55 years and in the bald parts of the scalp seen in sections treated with trichrome stain.

The results using the PAS technique are consonant with other findings in this study. Acid mucopolysaccharides cannot be demonstrated by this method and, of the PAS-positive material likely to be present in the skin, glycogen was eliminated by ensuring that the material was diastase-fast and glycolipids and sphingolipids were removed during the preparation of the sections, leaving only the glycoproteins for consideration.

Gersh & Catchpole (1949) have already shown that there is a thickening of the basement membrane with age by virtue of its increased content of glycoprotein and that an increased deposition of glycoprotein occurs within the ground substance in similar circumstances. Our findings are in agreement with these authors' and, by revealing an even thicker basement membrane in bald areas of the scalp, support the theory that the condition of the papillary layer of the dermis in baldness is an exaggeration, perhaps pathological, of the normal ageing process.

In the skin of the palm and sole, the papillary layer of the dermis gives a metachromatic reaction comparable to hairy parts of the skin although coarser collagenous fibres are found in that layer, but in view of its specialized function it would be unreasonable to expect the dermis of the palm and sole to conform either to the pattern of the rest of the skin or to the unusual circumstances found in the bald scalp. More accurate estimations of the increase in thickness of the collagenous fibres and basement membrane and of the reduction in the depth of the papillary layer will be included in a future publication.

SUMMARY

The ground substance of the papillary (subepidermal) layer of the dermis and the basement membrane have been studied in the skin from different regions of the body with particular reference to baldness.

Toluidine blue was employed to identify metachromatic elements and the PAS technique for the glycoproteins of the basement membrane. Collagen was stained by Masson's trichrome method.

A new technique is described involving digestion of the tissue sections with pepsin to free sulphated mucopolysaccharides from their combination with protein and allow them to be stained metachromatically. In material fixed within 12 hr. of death, metachromasia is present in the papillary layer of the dermis from all parts of the body except the bald scalp. Material for which fixation has been delayed for 36 hr. undergoes additional preliminary protein autolysis due to cathepsin A: thereafter, the application of the technique described reveals (*a*) that the release of the sulphated mucopolysaccharides from their combination with protein is thereby more complete, and (*b*) that in the papillary layer of the dermis there has been increased deposition of the sulphated mucopolysaccharides with age and especially in skin where hair has ceased to grow.

In those same areas, the PAS technique demonstrated marked thickening of the basement membrane, and trichrome staining a reduction in depth of the papillary layer with thickening of its collagen fibrils.

The conditions described for the papillary layer of the dermis in bald scalp may be considered as an exaggeration of the normal ageing process seen elsewhere in the skin.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Skin of chest from male aged 23; fixed within 12 hr. postmortem; shows pronounced metachromasia in papillary layer of dermis.

Fig. 2. Skin of bald scalp, male aged 52; fixed within 12 hr. postmortem; no metachromasia in the papillary layer.

Fig. 3. Skin of pubic region from same subject as in Fig. 2; fixed within 12 hr. postmortem; metachromasia present in papillary layer.

Fig. 4. Skin from bald part of scalp, male aged 52, fixation delayed until 42 hr. postmortem; pronounced metachromasia in papillary layer.

Fig. 5. Skin of abdomen from same subject and with same delay in fixation as in Fig. 4; pronounced metachromasia in papillary layer but less intense than in Fig. 4.

Plate 2

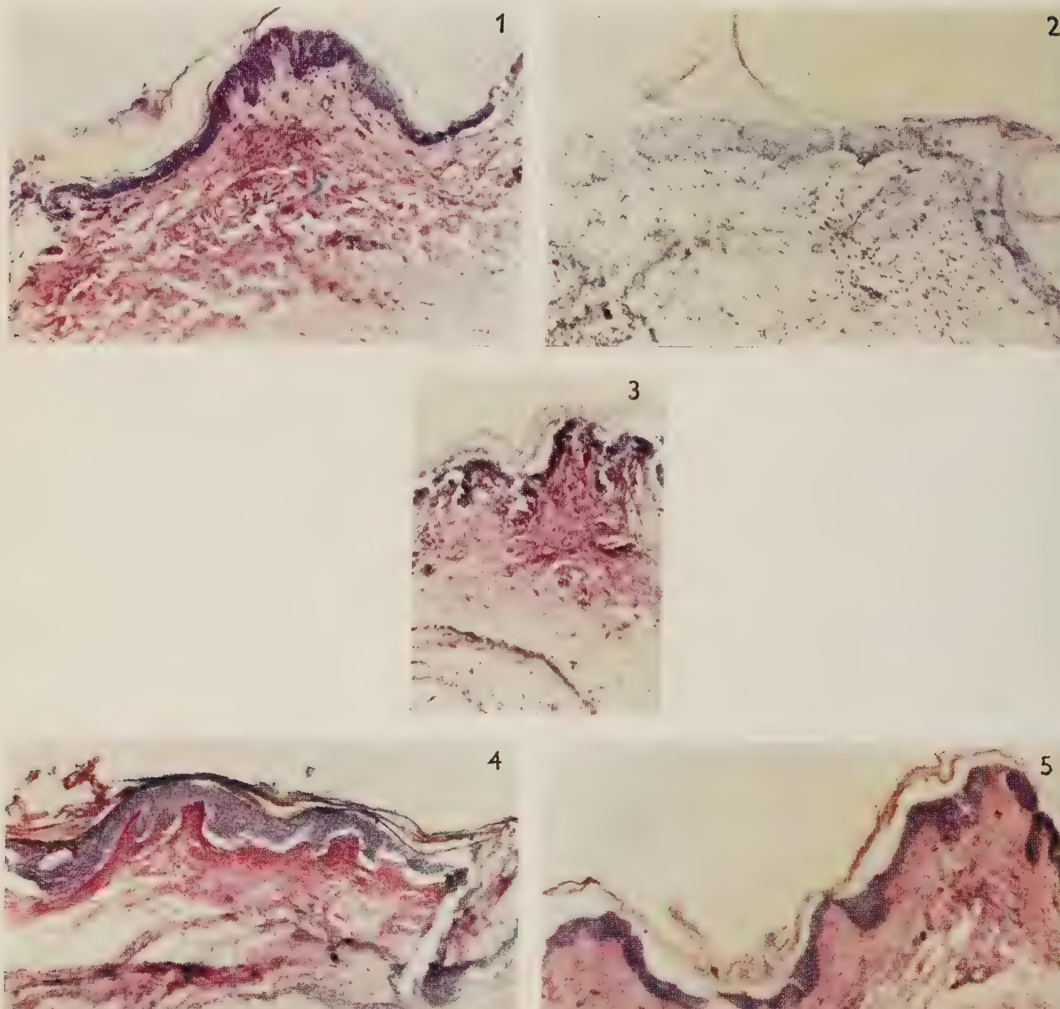
Fig. 6. Skin of bald scalp from same subject as in Pl. 1, fig. 2, trichrome stain; papillary layer of dermis shows marked reduction in depth due to encroachment of thick collagenous bundles of reticular layer.

Fig. 7. Skin of pubic region from same subject as in Pl. 1, fig. 3 and Pl. 2, fig. 6; trichrome stain; shows thick papillary layer containing fine collagenous fibres.

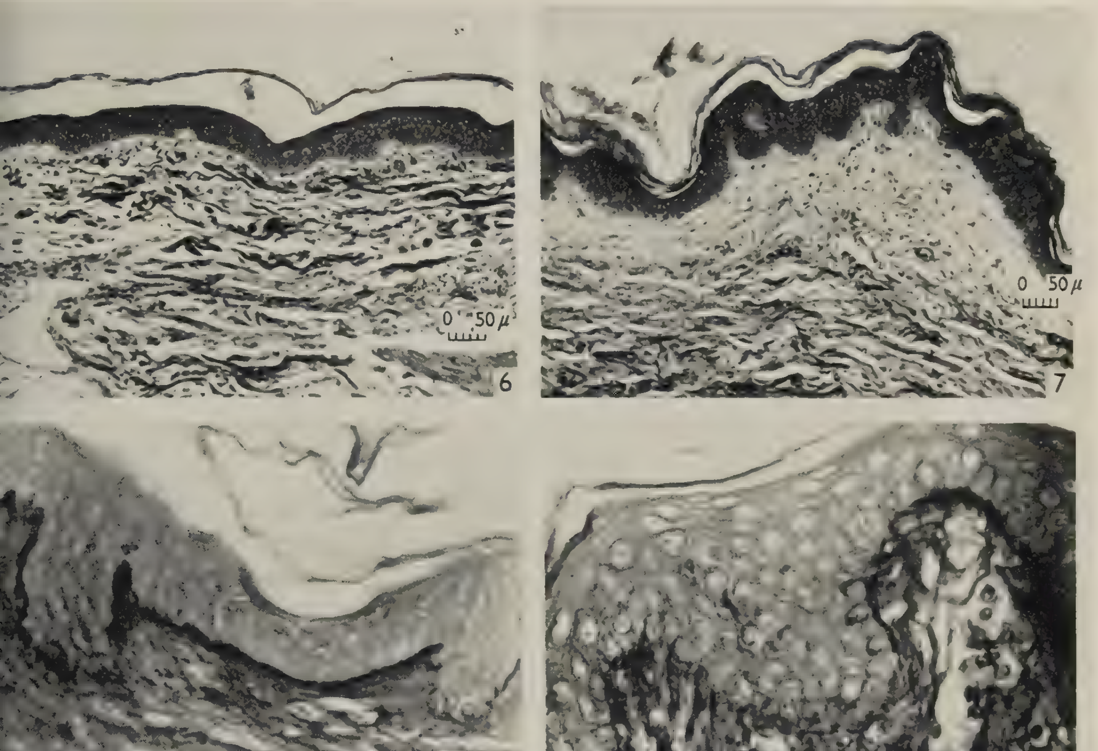
Fig. 8. Skin of chest from same subject as in Pl. 1, fig. 1; PAS; shows moderately thick but broken basement membrane; ground substance of papillary layer PAS positive.

Fig. 9. Skin of pubic region from same subject as in Pl. 1, fig. 3 and Pl. 2, fig. 7; PAS; very thin broken basement membrane.

Fig. 10. Skin of bald scalp from same subject as in Pl. 1, fig. 2 and Pl. 2, fig. 6; PAS; shows very thick continuous basement membrane.



SINGH AND McKENZIE—HISTOLOGY AND HISTOCHEMISTRY OF THE DERMIS



SINGH AND McKENZIE—HISTOLOGY AND HISTOCHEMISTRY OF THE DERMIS

THE STRUCTURE AND INNERVATION OF THE MYOTOMES OF THE LAMPREY

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In lower vertebrates, the trunk musculature is in the form of segmental myotomes, in which the muscle fibres lie parallel to the long axis of the body and are attached at their ends to connective tissue myosepta. Probably the most primitive arrangement of vertebrate somatic musculature is found in *Amphioxus*, where V-shaped myotomes are present along the entire length of the body. In cyclostomes, this simple segmental structure has been lost in the pre-otic region, but is present along the rest of the trunk, where it is not disturbed by the presence of paired fins as is the case in higher fishes. Other primitive features of the lamprey are the failure of the ventral and dorsal roots of the spinal nerves to unite after emerging from the spinal cord, and the absence of myelin sheaths in the peripheral nerves (Peters, 1960).

Grenacher (1867) showed that the myotomes of the lamprey are built up of a series of units (Kästchen), each of which contains two types of muscle fibre, parietal and central. This was confirmed by Maurer (1894) and Schiefferdecker (1911), who pointed out that the central muscle of a unit is in the form of plates rather than fibres. The peripheral nervous system has been studied by Tretjakoff (1927); and further important contributions to the innervation of the myotomes have been made by Johnston (1908), though his observations were incidental to a study of the cranial nerves. Little can be added to Maurer's (1894) account of the structure of the myotomes at the light microscope level, but the mode of innervation of the segmental muscle units is not described in these early investigations. It was therefore decided to re-examine the structure of lamprey myotomes, making a detailed study of their innervation, by the use of a silver-staining method, supplemented by cholinesterase staining and electron microscopy. The results are considered in the light of recent observations on the structure and function of muscle in the higher vertebrates.

MATERIAL AND METHODS

The trunk musculature of adult specimens of *Lampetra fluviatilis*, and of ammocoetes of *L. planeri*, was examined. For silver staining, material was fixed either in alcohol–formol–acetic (50 % alcohol, 90 ml.; formol, 5 ml.; glacial acetic acid, 5 ml.), or in 10 % formalin, and was stained by a silver nitrate–egg albumen method (Peters, 1958).

For the demonstration of sites of cholinesterase activity, small blocks of tissue were fixed for 1 hr. in 10 % formalin, and frozen sections cut at 30–60 μ. These were stained either with a modification of Koelle's (1950) acetylthiocholine technique, or with the azo-dye method of Lewis (1958); both procedures gave good results.

For electron microscopy, small pieces of tissue were fixed for 1 hr. at 4° C. in 1 %

osmic acid adjusted to pH 7·2 with a veronal acetate buffer (Palade, 1952). The tissue was then washed in 10% alcohol, dehydrated, and embedded in 1:8-methyl-butyl methacrylate. Thin sections were cut with a glass knife, using a Porter–Blum microtome, and examined in a Metropolitan-Vickers, E.M. 6, electron microscope.

RESULTS

Structure of the myotomes

In the lamprey, each myotome is folded behind the one in front, and in horizontal sections a myotome lies obliquely with its medial border anterior relative to its lateral border. Thus, in a transverse section through the animal, parts of three to five myotomes are visible, the most anterior of which lies next to the skin, with those posterior to it lying successively nearer to the midline (Text-fig. 1A).

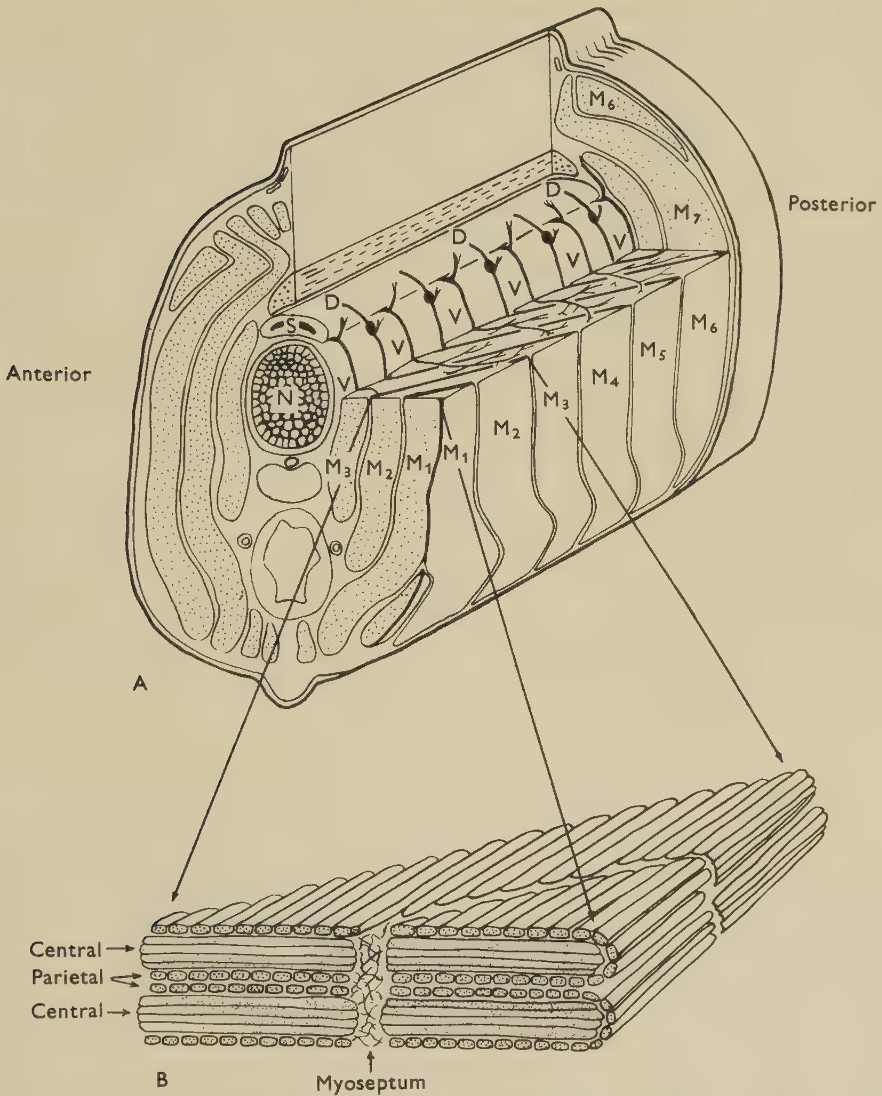
Silver-stained sections show that a myotome is composed of a series of horizontally arranged units, stacked one above the other. Each unit extends throughout the myotome from its lateral to medial, and cephalic to caudal extents, and usually contains six horizontal layers of muscle, though sometimes only five are present (Text-fig. 1B).

The muscle of the dorsal and ventral layers of each unit differs from that of the central layers in its morphology and innervation, and is composed of a series of longitudinally oriented muscle fibres (parietal muscle fibres), each 20–30μ thick, lying side by side. Around the lateral aspect of the unit, where the myotome abuts on the skin, these two layers are continuous with each other, but elsewhere the central layers are exposed and project beyond the limits of the parietal layers (Pl. 1, fig. 2).

Each of the central layers is 40–60μ thick, and appears to be a continuous sheet of muscle, for in sections stained by Wilder's reticulin method, no connective tissue partitions can be seen subdividing the sheets internally, although a thin layer of connective tissue is present between adjacent layers of a unit. That each of the central layers is a complete sheet can be seen clearly in transverse sections through ammocoete myotomes, but in similar sections of an adult lamprey the central plates are sometimes broken up (Pl. 1, fig. 4), probably as a consequence of shrinkage during preparation. Furthermore, it is possible to obtain horizontal sections passing through a single central layer over the whole extent of a myotome, and these show no apparent subdivision of the sheet into component fibres. Therefore each central layer can probably be considered as a single muscle plate.

While the central muscle plates of each unit have only a thin network of connective tissue between them, each parietal muscle fibre is surrounded by a thick layer of connective tissue which binds it firmly to adjacent parietal fibres in its own and the neighbouring unit (Pl. 1, fig. 4), and at the edge of a unit is often continuous with the subcutis of the skin. Thus, though the central plates may sometimes become separated during processing of the tissue, each layer of parietal fibres always remains intact.

While the parietal muscle fibres are well formed and clearly visible in the adult lamprey, in a 10 cm. ammocoete they are less distinct and form only a thin layer (2–3μ thick) on the outside of a unit. However, the thickness of the parietal layers



Text-fig. 1. Diagrams showing the arrangement, and composition, of lamprey myotomes. A, Portion of the body has been cut away to show the arrangement of the myotomes (M_1–M_7). These are W-shaped, and are folded obliquely one behind the other. The spinal cord (S) lies above the notochord (N), and the ventral (V) and dorsal (D) nerve roots emerge alternately from each side of the spinal cord. Each ventral root divides into dorsal and ventral rami which run on the medial surface of the myotomes; some of the nerve fibres from the ventral roots pass into the myotomes, as shown in the horizontal section, to innervate the parietal muscle fibres. B, Enlargement of parts of two myotomes (M_1 and M_2 in A), showing the structure of the muscle units which make up the myotomes. Each unit extends through the whole width of a myotome and consists of (usually) four central plates bounded, except at the medial and myoseptal surfaces, by a single layer of parietal muscle fibres. (See text.)

increases as the animal grows, so that in the adult each parietal layer approaches the thickness of a central muscle plate (Pl. 1, fig. 3).

After silver staining, the myofibrils of the parietal muscle fibres appear thicker and more widely separated than those of the central muscle plates (Pl. 1, fig. 3). (This is clearly indicated in the diagrams of Maurer (1894) and Schiefferdecker (1911).) In electron microscope sections, these differences in thickness of the myofibrils are not apparent, but the myofibrils of the parietal muscle (Pl. 3, fig. 11) are more widely separated by sarcoplasm than those of the central plates, and the parietal muscle sarcoplasm contains relatively larger numbers of mitochondria (Pl. 2, fig. 9; Pl. 3, fig. 11). In addition, the sarcoplasm of both types of muscle contains electron dense, homogeneous bodies (Pl. 2, fig. 9; Pl. 3, fig. 11), and these are more numerous in the parietal muscle where they are often concentrated under the sarcolemma (Pl. 3, fig. 11).

Both parietal fibres and central plates are attached to the myosepta by myotendinous junctions which do not appear to differ in structure from those described in other vertebrates by Schwarzacher (1959) and Muir (1961). At a myotendinous junction, the sarcolemma displays numerous invaginations (Pl. 2, fig. 10, J) from which emerge the collagen fibrils of the myoseptum, while the myofilaments appear to be attached to the inner surface of the invaginated sarcolemma. The parietal fibres taper as they approach a myoseptum (Pl. 1, fig. 2), so that the area occupied by their myotendinous junctions is not extensive; the invaginations of their junctions are, however, deeper than those of the central muscle plates which end bluntly by myotendinous junctions extending through the entire width of the myotome from its lateral to medial extent.

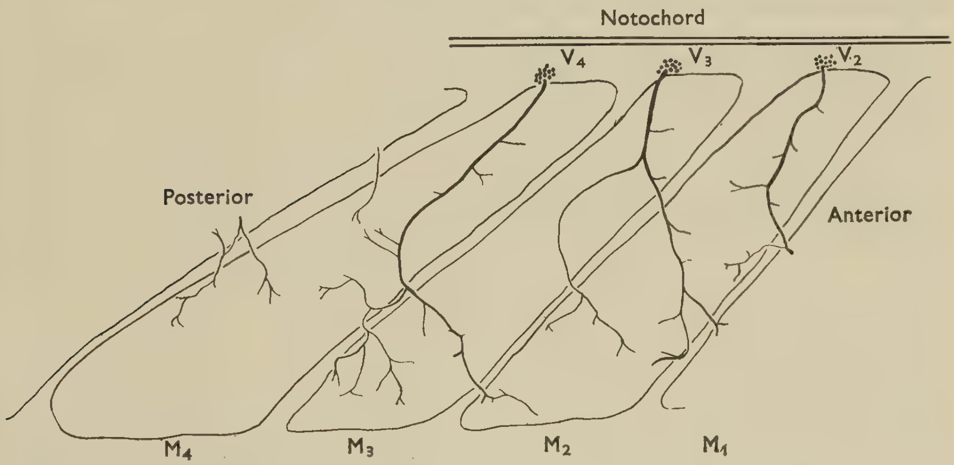
Innervation of the myotomes

In the lamprey, the pairs of dorsal and ventral nerve roots emerge alternately from the spinal cord (Text-fig. 1A) and remain separate throughout their peripheral course. The peripheral nerves are unmyelinated, but each axon is surrounded by a sheath which is one Schwann cell thick (Peters, 1960). At this level, most of the nerve fibres fall within the size range 3 to 6μ, though some may be as large as 10μ in diameter. The dorsal roots run through myosepta to the skin, and branches to the myotomes have not been seen. Each ventral root divides into dorsal and ventral rami which course over the inner surface of the myotomes (Text-fig. 1A), giving branches which either enter the myotomes to run between the muscle units, or sweep round the edges of the myotomes to enter the myosepta on each side. The course of these nerves is most easily followed in horizontal sections.

In large ammocoetes, it can be seen that one, and less frequently two, nerve fibres from the ventral roots enter the myotomes between each of the muscle units, so that a nerve fibre is sandwiched between every adjacent pair of parietal muscle layers. Nerve fibres in this position proceed laterally, and give branches which innervate both layers of parietal muscle fibres. They do not remain within the same myotome, however, for having innervated the medial half or two-thirds of a pair of parietal muscle layers in the myotome, a nerve then crosses the anterior myoseptum to enter the myotome in front, again coming to lie between and to supply two adjacent parietal muscle layers in the lateral portion of that myotome (Text-fig. 2). Some-

times a nerve may cross over into yet another myotome to innervate parietal muscle fibres at the extreme lateral margin of this myotome. The distribution of some of these nerve fibres is shown in Text-fig. 2, which is based on the paths of nerve fibres in a 10 cm. ammocoete of *L. planeri*. The distribution of these nerve fibres is the same in adults of *L. fluviatilis*, but while the nerves give off relatively few branches in this ammocoete, in the adult the branches are more numerous and they divide frequently to give off fine collaterals which produce an extensive network between adjacent muscle units (Pl. 1, fig. 5). Some of these fine branches appear to terminate in expansions on the surface of the parietal muscle fibres, but no well-defined end-plates have been observed in the silver-stained sections.

The nerve branches which sweep round the edge of a myotome and enter the myosepta are best observed in transverse sections, where it can be seen that they



Text-fig. 2. Diagram to show the distribution of the nerves which enter the myotomes and run between the muscle units. Nerves from the ventral roots (V_2–V_4) enter the corresponding myotomes (M_1–M_4) and innervate parietal muscle fibres in the medial part of this myotome before crossing the anterior myoseptum to pass into the lateral part of the myotome in front. Sometimes one nerve may innervate parietal muscle fibres in three different myotomes; for example, V_4 innervates M_4, M_3 and M_2. This diagram is based on reconstructions of the paths of nerves in a 10 cm. ammocoete of *L. planeri*.

form an extensive network over the ends of the central muscle plates of the units. They do not extend beyond the edges of the central plates, however, so that while each muscle unit is surrounded by nerve networks on all sides, no nerve fibres are found within the units.

The two histochemical procedures used to demonstrate sites of cholinesterase activity give similar results on lamprey myotomal muscle. In the frozen section illustrated in Pl. 1, fig. 6, which has been cut in a parasagittal plane and stained with the acetylthiocholine technique, the muscle units are outlined by zones of stain. Considerable diffusion has occurred, but it is possible to recognize that the enzyme is confined to the myoseptal margins of the units, and to the parietal fibres all along their length; the bulk of the central plates is free from stain. When diffusion

is minimized by reducing the incubation time (Pl. 2, fig. 7) it is clear that most of the staining along the parietal fibres can be attributed to the nerve fibres running between the units (Pl. 2, fig. 7, N). With the azo-dye method, it is possible to reduce nerve staining considerably, and these preparations show small areas of intense staining at intervals along the parietal fibres. The supposition that they represent neuromuscular junctions is supported by electron microscopy (Pl. 3, fig. 11).

Cholinesterase is confined to the myoseptal margins of the central muscle plates (Pl. 1, fig. 6; Pl. 2, fig. 7). Part of this reaction may be due to a myotendinous response as at most vertebrate neuromuscular junctions (see Gerebtzoff, 1959), but it may be observed in Pl. 2, fig. 8, that small circumscribed zones of intense staining (indicated by arrows) are present towards the periphery of the stained region. These may well be the sites of motor end-plates, since electron microscopy shows that the end-plates (Pl. 2, fig. 10, E) are situated just short of the actual edge of the plate, at the sides of the myotendinous junctions (Pl. 2, fig. 10, J).

The Schwann cell sheath is absent at myoneural junctions, and at this point the basement membrane on the outside of the sheath blends with that of the sarcolemma (Pl. 3, fig. 12, L). Apart from this common layer derived from the two basement membranes, the axolemma and sarcolemma come into close proximity and are separated by a distance of only 500–700 Å. (Pl. 3, fig. 12; Pl. 4, fig. 13). The axoplasm of a nerve ending contains numerous vesicles, 250–500 Å. in diameter (Pl. 3, fig. 12; Pl. 4, figs. 13, 14, V), and a higher density of mitochondria than is found elsewhere in the axoplasm (Pl. 3, fig. 12; Pl. 4, fig. 14, M). These features also characterize the neuromuscular junctions in other animals (e.g. Robertson, 1957).

The parietal muscle is innervated by the nerves running between the muscle units, and in electron microscope sections the neuromuscular junctions on a parietal muscle fibre are found on the side of the fibre adjacent to the neighbouring muscle unit (Pl. 3, fig. 11). A neuromuscular junction typical of a parietal muscle fibre is illustrated in Pl. 3, fig. 12, and the Schwann cell sheath can be seen to be absent in two regions, E_1 and E_2, although the subjacent sarcolemma shows none of the complex infoldings that characterize the subneural apparatus of higher vertebrates (Couteaux, 1955). Sometimes a single nerve fibre may form neuromuscular junctions on two adjacent parietal muscle fibres, as in the case of the nerve illustrated in Pl. 3, fig. 11, which forms neuromuscular junctions at the sites indicated by arrows, although at the magnification of the micrograph it is not possible to see them clearly.

In their morphology, most of the myoneural junctions on the central muscle plates resemble those of the parietal fibres where the subneural apparatus is either absent or poorly developed (Pl. 3, figs. 11, 12). At the myoneural junctions illustrated in Pl. 4, fig. 13, the sarcolemma adjacent to the nerve shows only one small invagination (arrow). In the few instances in which a well-defined subneural apparatus has been observed at the neuromuscular junction on a central plate, it has the same form as that described at reptilian (Robertson, 1956) and human (De Harven & Coers, 1959) neuromuscular junctions. The sarcolemma is thrown into numerous invaginations which are lined by the sarcolemmal basement membrane, while the axolemma does not enter these invaginations but bridges over them. However, in the lamprey these invaginations are more irregular in their arrangement than those of either the reptilian or human myoneural junction where they are evenly spaced.

In the lamprey, axons terminating on either parietal or central muscle usually lie in shallow depressions of the sarcolemma, but occasionally on a central plate the nerve fibre approaching a neuromuscular junction lies in a well-defined synaptic gutter (Pl. 2, fig. 10).

From this description it is clear that there is considerable variation in the structure of lamprey myoneural junctions, particularly in the arrangement of the sarcolemma, which may sometimes form a well-defined subneural apparatus, but more frequently shows very few or no invaginations. A gradation between these two extremes is often seen in electron microscope sections through the site of innervation of a central muscle plate, where many individual myoneural junctions may be present in one section. These junctions also vary in other ways. Sometimes, as in Pl. 4, fig. 14, the axon is small and packed with vesicles, while in other cases, as in Pl. 4, fig. 13, a large axon is bare over a small area and the vesicles are confined to that region. The myoneural junction in Pl. 4, fig. 13, is one of three formed by the same large axon (diameter $10\,\mu$) on the side of one muscle plate. All three junctions have the same form and are separated from each other by a distance of 15–$20\,\mu$. Thus, in contrast to the parietal muscle fibres, a section through the site of innervation of a central muscle plate may show different forms of neuromuscular junctions, and as many as seven different points of innervation have been observed on a single muscle plate in the same electron microscope section. This would seem to indicate that despite the apparent continuity of each central sheet, it is innervated along the whole length of its myoseptal margin.

DISCUSSION

The muscle units in *Lampetra fluviatilis* and in *L. planeri* have the same structure, and this appears to be constant in the lamprey, since in addition to the above species it has been described by Grenacher (1867) for *Petromyzon marinus*, by Johnston (1908) for *P. dorsatus*, and by Peachey (1960*b*) for *P. branchialis*. In his studies on *L. fluviatilis*, Maurer (1894) considered that the parietal muscle was formed by an anastomosing network of fibres, a view supported by Schiefferdecker (1911), but we are unable to confirm this finding as both light and electron microscopic preparations show that the parietal muscle is composed of individual fibres.

A more difficult concept is the organization of the muscle of the central layers of a unit into individual sheets. Proof of this must depend on the failure to demonstrate any vertical fibrous partitions or sarcolemmal sheets within the central muscle layers, and since these have not been observed in our preparations we must conclude that each central layer consists of a single, continuous sheet of muscle, invested in a separate sarcolemmal sheath, with no subdivision into smaller components. The only other instance known to us of vertebrate muscle occurring in sheets is in *Amphioxus*, where Grenacher (1867) described the myotomes as being built up of muscle plates; this has recently been supported by Peachey (1960*a*) from electron microscope studies.

The two types of muscle in the lamprey have been shown to differ in their mode of innervation, the parietal muscle fibres being innervated along their length, while the central plates are innervated at both ends in the myoseptal regions.

Johnston (1908) states that the fine terminal branches of the nerves to the parietal muscle fibres are 'moderately slender, slightly varicose fibres which show no signs of any special end organ'. A similar conclusion is reached by Tretjakoff (1927), although he shows some of the terminal branches of the nerves ending in knob-like expansions. Our own preparations show no such varicosities of these fine terminal branches and no structures that could be termed nerve terminals are visible in them. As pointed out by Johnston (1908), although it is clear that the distribution of one nerve fibre to two and sometimes three myotomes appears to be the rule, no explanation for such a distribution is apparent.

The central muscle plates appear to be innervated by the nerves which sweep round the edges of the myotomes into the myosepta, and the myoneural junctions on these central plates lie at the edges of their myoseptal margins. Johnston (1908) also describes the myoseptal nerves branching to form end-plates in this region. A similar form of innervation occurs in the myotomes of a number of other lower vertebrates (Mackay & Peters, 1961), in which the muscle fibres are also innervated at both ends. The site and form of the endings may, however, vary somewhat: in *Xenopus* tadpoles, for instance (Mackay, Muir & Peters, 1960), the neuromuscular junctions are related to the sarcolemma within or between the invaginations at the myotendinous junction, and not to its side as in the lamprey.

The rather diffuse staining obtained after the histochemical demonstration of cholinesterase in lamprey muscle may be a consequence of the absence of a well-developed subneural apparatus such as is found in the higher vertebrates (e.g. Robertson, 1956; De Harven & Coers, 1959). In electron microscope sections, a subneural apparatus is often not seen at the myoneural junction, but this may be because the apparatus is poorly developed so that many sections do not pass through sarcolemmal infoldings. Absence of a subneural apparatus has been described in the neuromuscular junctions of leg muscles of the wasp (Edwards, Ruska & De Harven, 1958), and in vertebrate smooth muscle (Caesar, Edwards & Ruska, 1957). When present, however, the subneural apparatus in the lamprey resembles that of higher vertebrates in its basic structure, though the infoldings are relatively few and are usually more shallow and irregular in their arrangement.

The reason for the existence of two types of muscle in the lamprey is obscure, though the differences in morphology and innervation between these two types indicate that they subserve different physiological roles. The parietal fibres have relatively more sarcoplasm, more nuclei, and more extensive innervation than the central muscle plates, and these characteristics are also shown by muscle fibres in higher vertebrates which have been demonstrated to have a tonic or postural function. On the other hand, the structure of the central muscle plates is more akin to that of the tetanic or twitch fibre of higher vertebrates. (For a review of this subject, see Mackay, 1961.)

Some information is available on the other commonly occurring cyclostome, *Myxine*, where two varieties of muscle fibre have also been described (Maurer, 1894; Cole, 1907). Again the myotomes are built up of muscle units, but fibres corresponding to the lamprey parietal fibres are confined almost entirely to a single layer on the ventral aspect of a unit. The second form of muscle in *Myxine*, forming the bulk of the units and corresponding to the central plates of the lamprey, is described as

occurring in fibres rather than sheets. These two types of muscle in *Myxine* have very recently been studied electrophysiologically by Andersen & Jansen (personal communication), who reach the tentative conclusion that the two histological types may have functional properties corresponding to the fast and slow muscle fibres of the frog (Kuffler, 1953).

SUMMARY

The segmental myotomes in the lamprey are built up of a series of horizontally arranged muscle units, stacked one above the other. Each unit extends throughout the entire horizontal plane of a myotome, and usually consists of six layers; four central muscle plates are bounded dorsally, laterally and ventrally by a single layer of closely packed parietal muscle fibres.

Cholinesterase activity is found along the length of the parietal muscle fibres, whereas it is confined to the myoseptal margins of the central plates. The parietal fibres are innervated at points along their length by nerves which enter the myotomes between the muscle units to form a plexus which thus lies between adjacent layers of parietal muscle. Having supplied the parietal fibres in the medial portion of one myotome, a nerve crosses a myoseptum to enter the myotome in front, and may even reach a third myotome, supplying parietal muscle fibres in the lateral portions of the second and third myotomes. The central muscle plates are innervated at both myoseptal margins by ventral root fibres that enter the myosepta. Nerves do not penetrate into the individual muscle units.

The sites of innervation are confirmed by electron microscopy, which shows that at the myoneural junctions the folds of the sarcolemma are shallow and irregular in their arrangement. At such junctions, the nerve fibres lose their sheaths and the axoplasm contains numerous mitochondria and vesicles.

It is suggested that the central muscle plates may have a twitch and the parietal fibres a tonic function.

We wish to thank Prof. G. J. Romanes for his interest and advice in the course of this work. The electron microscope, which is on permanent loan from the Wellcome Foundation, was maintained by Mr G. Wilson.

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EXPLANATION OF PLATES

These figures are of adult specimens of *L. fluviatilis.*

PLATE 1

Fig. 1. Transverse section of part of a myotome (M) adjacent to the notochord (N); the myotome is composed of a series of muscle units (U). Silver stain, ×40.

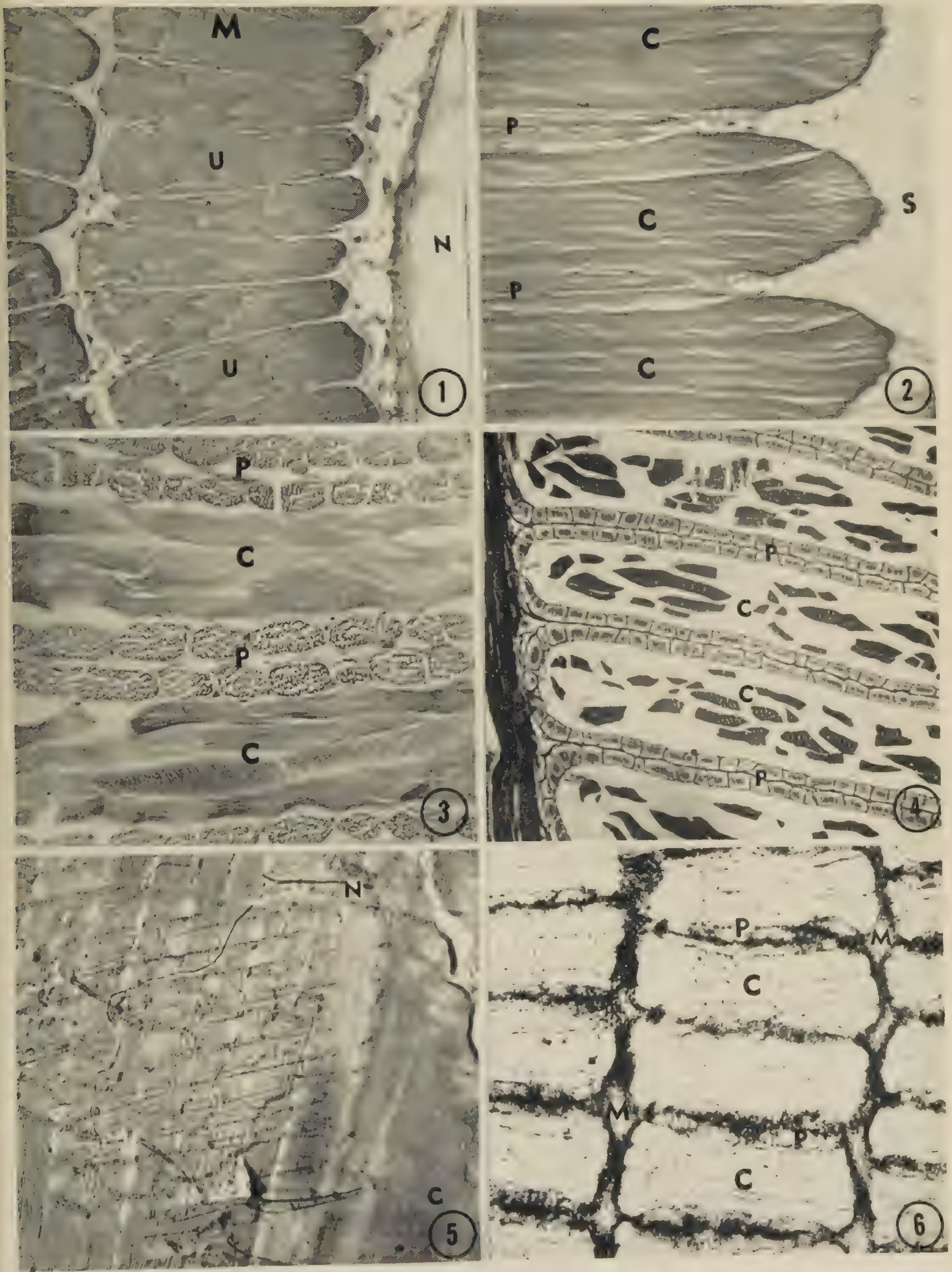
Fig. 2. Parasagittal section of part of a myotome. The parietal muscle fibres (P) taper as they approach the myoseptum (S) and terminate short of the central muscle plates (C) which end bluntly. Silver stain, ×110.

Fig. 3. Transverse section of part of a myotome. The myofibrils of the parietal muscle fibres (P) are more widely separated than the finer myofibrils of the central muscle plates (C). Silver stain, ×120.

Fig. 4. Transverse section of muscle units stained by Wilder's reticulin method. The parietal muscle fibres (P) extend around the lateral surfaces of the muscle units where these come in contact with the skin (on the left). In contrast to the central muscle plates (C) the parietal muscle fibres are bound tightly together by connective tissue. In this section the central muscle plates are fragmented, so that the arrangement of the parietal fibres is more apparent. ×90.

Fig. 5. Horizontal section through the myotomal muscle of an adult lamprey, showing a nerve fibre (N) branching over the surface of the parietal muscle fibres. At the edge of the section, part of a central muscle plate (C) is visible. Silver stain, ×100.

Fig. 6. Parasagittal section of the myotomal muscle stained by the acetylthiocholine method for cholinesterase; the preparation is overstained and some diffusion has occurred. The enzyme is found along the length of the parietal muscle fibres (P), but on the central muscle plates (C) is confined to the regions adjoining the myosepta (M). ×45.



PETERS AND MACKAY—STRUCTURE AND INNERVATION OF MYOTOMES OF THE LAMPREY

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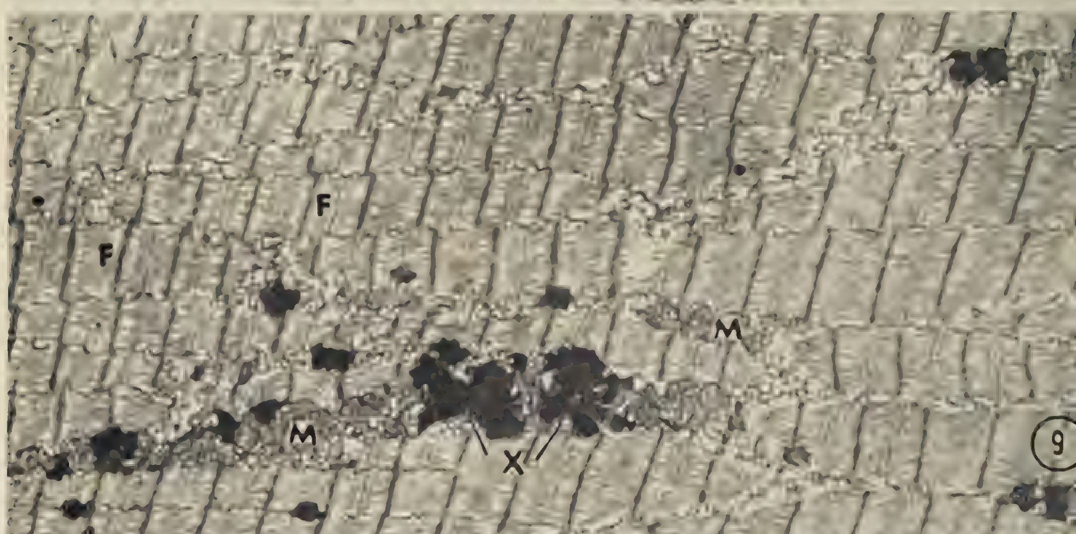

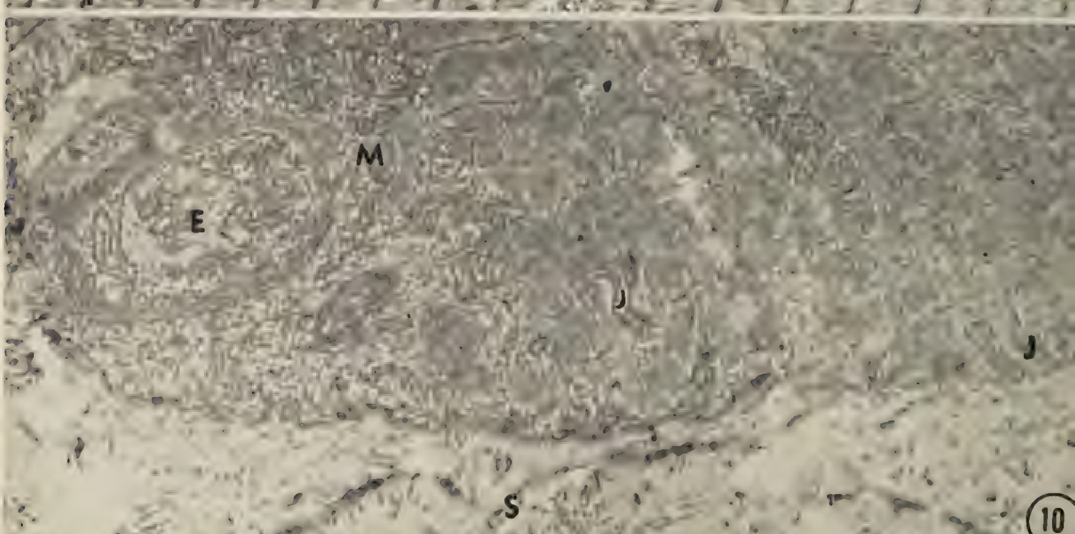

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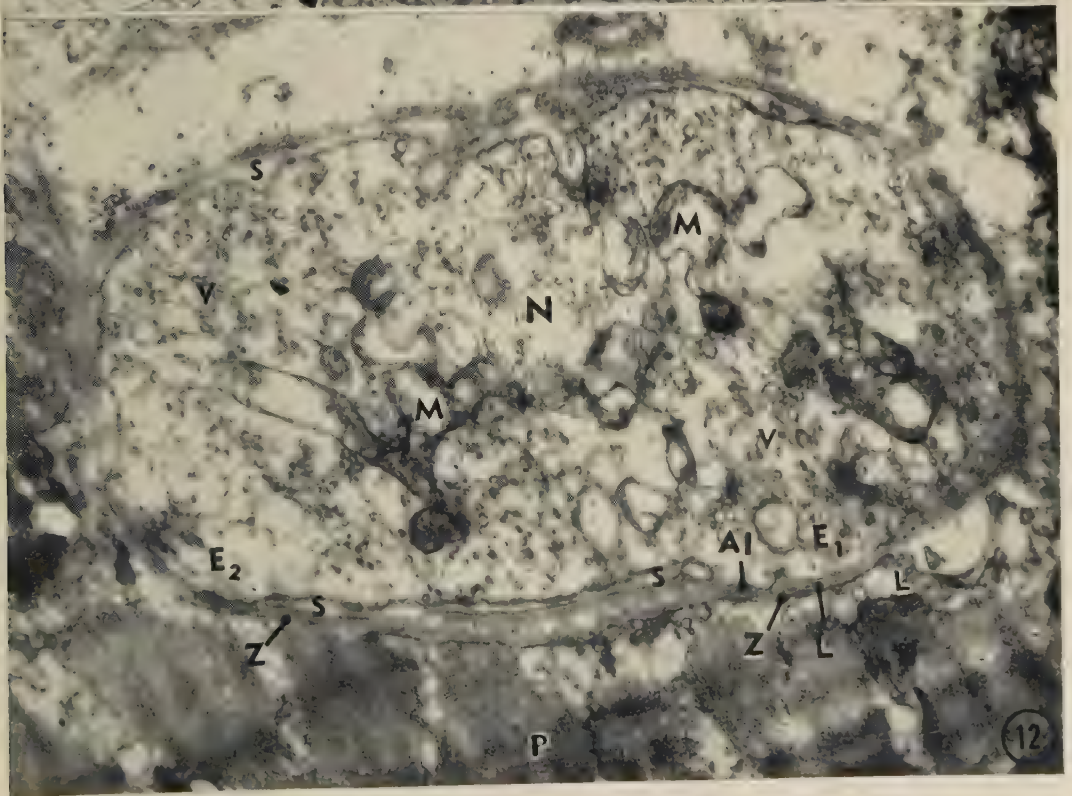

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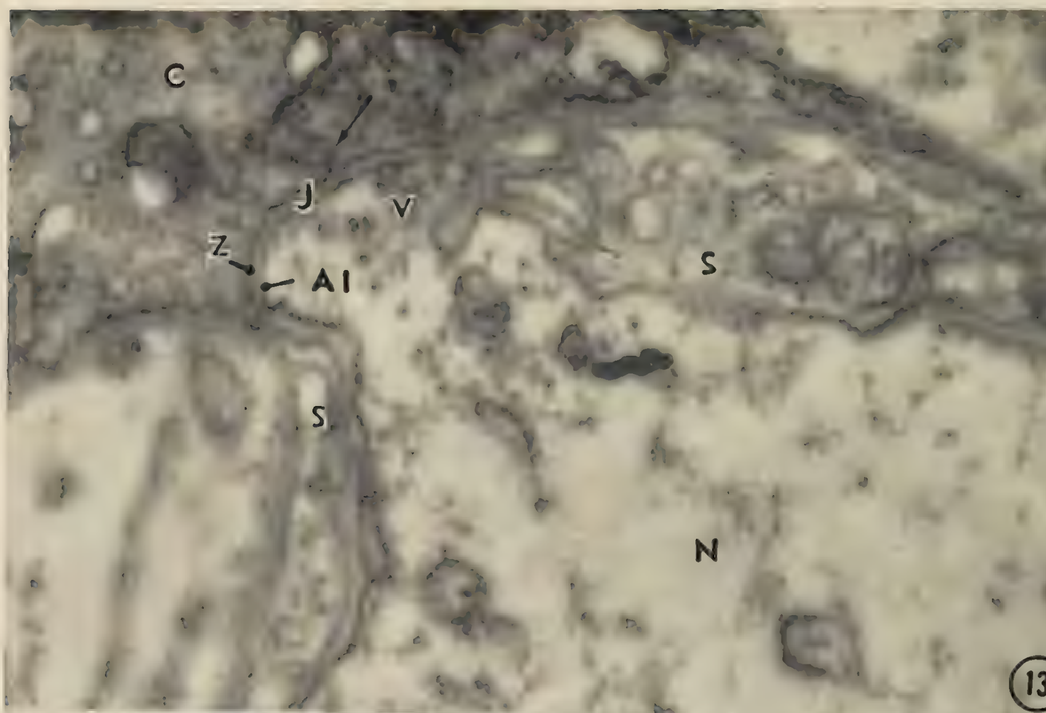

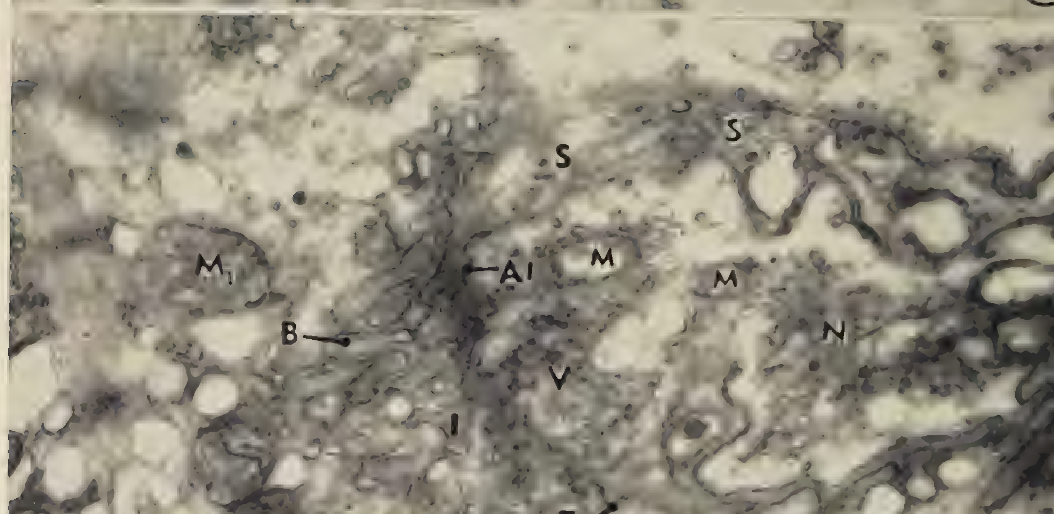

PETERS AND MACKAY—STRUCTURE AND INNERVATION OF MYOTOME˷ OF THE LAMPREY

PLATE 2

Fig. 7. Parasagittal section of the myotomal muscle stained by the acetylthiocholine method. Cholinesterase is present at the ends of the central muscle plates (C) where these adjoin the myosepta (X). The apparent staining of the parietal muscle is due to cholinesterase located in the nerve fibres (N) that run between the adjacent layers of these fibres. ×100.

Fig. 8. Part of a central muscle plate in the myoseptal region; acetylthiocholine method. Most of the cholinesterase activity is the result of a myotendinous reaction (T), but small areas of intense staining (arrows) are present on the sides of this region. ×610.

Fig. 9. Electron micrograph of a central muscle plate. The myofibrils (F) are closely packed together, so that there is little sarcoplasm between them. The mitochondria (M) are fewer in number than in the parietal muscle fibres (Pl. 3, fig. 11). Note the osmiophilic bodies (X) between the myofibrils. ×8,000.

Fig. 10. Electron micrograph of part of the myotendinous region of a central muscle plate. A nerve ending (E) indents the muscle plate at the side of the myotendinous junction, where connective tissue fibrils of the myoseptum (S) are inserted into the invaginations (J) of the sarcolemma. Large numbers of mitochondria (M) are present in the sarcoplasm in the region of the nerve ending (E). ×5,000.

PLATE 3

Fig. 11. Electron micrograph of parts of two parietal muscle fibres (P_1 and P_2), one of which (P_2) contains a nucleus (Nu). The sarcoplasm between the myofibrils (F) is more extensive than in the central muscle plates (fig. 9), and contains more mitochondria (M). Between the two parietal muscle fibres is a nerve (N), packed with vesicles and mitochondria, which forms neuromuscular junctions with two parietal muscle fibres at the places indicated by arrows. Note the osmiophilic bodies (X) between the myofibrils and under the sarcolemma. ×8,000.

Fig. 12. Electron micrograph of a myoneural junction on a parietal muscle fibre (P). The nerve fibre (N) is packed with vesicles (V) and mitochondria (M). In two regions (E_1 and E_2) the Schwann cell sheath (S) is absent, so that the axolemma (Al) is only separated from the sarcolemma (Z) by a homogeneous layer (L) formed from the basement membranes on the outsides of the axolemma and sarcolemma. Note the absence of a subneural apparatus. ×24,000.

PLATE 4

Fig. 13. Electron micrograph of a myoneural junction (J) on a central muscle plate (C). This is one of three junctions formed by a large nerve fibre (N), 10μ in diameter. The axoplasm of the junctional region contains a localized concentration of vesicles (V), and the Schwann cell sheath (S) is absent, so that the axolemma (Al) comes into close proximity to the sarcolemma (Z) which has a small invagination (arrow). ×32,000.

Fig. 14. Electron micrograph of a myoneural junction on a central muscle plate (C). At the junction the nerve fibre (N) contains numerous vesicles (V) and mitochondria (M), and the Schwann cell sheath (S) is absent so that the axolemma (Al) comes into close proximity to the sarcolemma (Z). The sarcolemma (Z) has numerous invaginations (I) which are irregular in their arrangement and are lined by the basement membrane (B) of the sarcolemma. Numerous mitochondria (M_1) are situated in the sarcoplasm subjacent to the junctional region. ×33,000.

THE DURA MATER AT THE CRANIO-VERTEBRAL JUNCTION

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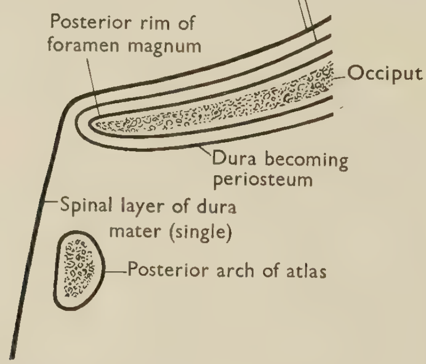
Some well known text-books of anatomy (Cunningham, 1951; Buchanan, 1953; Gray, 1958) draw a distinction between the cranial and spinal parts of the dura mater, stating that the former is composed of two layers, the latter of only one. At the foramen magnum it is said that the outer layer of the cranial dura mater blends with the periosteum of the skull while the inner layer of the cranial dura becomes the spinal theca. These statements imply an arrangement as shown diagrammatically in Text-fig. 1.

A study of the dura mater during surgical operations and from specimens from the post-mortem room has led us to believe that the above statements are incorrect.



Text-fig. 1. Diagrammatic representation of the arrangement of the dura mater at the cranio-vertebral junction according to some well known text-books of anatomy.

SURGICAL OBSERVATIONS

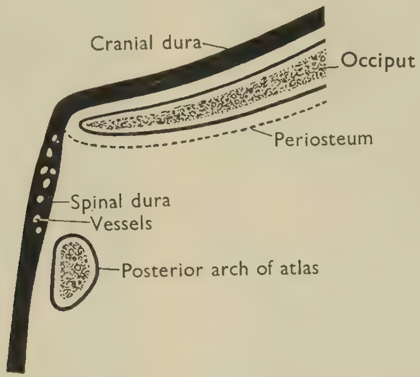
At surgical operations on the region of the cranio-vertebral junction, an instrument, such as a curved Adson director, can readily be passed from the spinal canal into the posterior fossa along the outer surface of the dura mater. Removal of the posterior margin of the foramen magnum and upper cervical laminae reveals a continuous dural membrane from the posterior fossa down into the spinal canal. At the edges of the foramen magnum there is only a flimsy attachment of the dura to the bone. Examination of the dura mater after it has been opened shows that the upper inch or so of the spinal theca is thicker than the continuation of the

membrane above or below. This thickened part of the spinal theca contains many small vessels within the membrane (Pl. 1, figs. 1, 2).

At operation one of us (L.C.R.) has demonstrated on many occasions that both the cerebral and spinal parts of the dura mater may be split into two layers with a scalpel: with care each layer may be further split into layers. This separation of the dura mater into layers is clearly artificial.

EXAMINATION OF SPECIMENS FROM THE POST-MORTEM ROOM

After one of us (L.C.R.) had many times observed the above facts in the operating room we examined specimens of the cranio-vertebral junction obtained at post-mortem. We were able to make similar observations on these specimens and have verified them histologically.



Text-fig. 2. Diagrammatic representation of the arrangement of the dura mater at the cranio-vertebral junction as revealed by our observations.

HISTOLOGICAL OBSERVATIONS

Close examination fails to substantiate the statement that the cerebral dura mater is composed of two layers. Its two surfaces are closely united except where they are separated by vascular channels. Histological examination of the membrane between these vascular channels shows interwoven collagen fibres without any semblance of a bilaminar arrangement. Vascular channels in parts of its substance do not make it a bilaminar structure. Sections of it from above and below the cranio-vertebral junction are essentially similar, and centrally placed vascular channels occur in the spinal as well as in the cranial dura.

At the cranio-vertebral junction, histological examination shows that the whole of the cerebral dura mater passes down into the spinal canal. At this position there is no formed layer passing from the dura mater on to the outer surface of the bones around the rim of the foramen magnum (Pl. 2, figs. 3–5).

We suggest the arrangement of the dura at the cranio-vertebral junction would correctly be depicted as in the diagram (Text-fig. 2).

SUMMARY

The intracranial and spinal parts of the dura mater are composed of a single continuous membrane. Both parts may readily be split into two or more layers by the surgeon in the operating room. Just below the foramen magnum the spinal theca is thick and vascular.

We are grateful to the Department of Medical Illustration of the Welsh National School of Medicine at the Cardiff Royal Infirmary for the photographs and diagrams.

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EXPLANATION OF PLATES

Plate 1

Figs. 1, 2. Photographs of the cranio-vertebral junction and upper end of the theca just below the foramen magnum. The thickening of the dura in this region is clearly seen. The arrows indicate the rim of the foramen magnum.

Plate 2

Figs. 3–5. Microscopical appearances of the dura mater at the cranio-vertebral junction.

Fig. 3. Anteriorly in median sagittal plane.

Fig. 4. Posteriorly in median sagittal plane (i.e. through squamous part of occipital bone and posterior arch of atlas).

Fig. 5. In para-median sagittal plane (i.e. just lateral to the odontoid process and just medial to the atlanto-occipital joint).

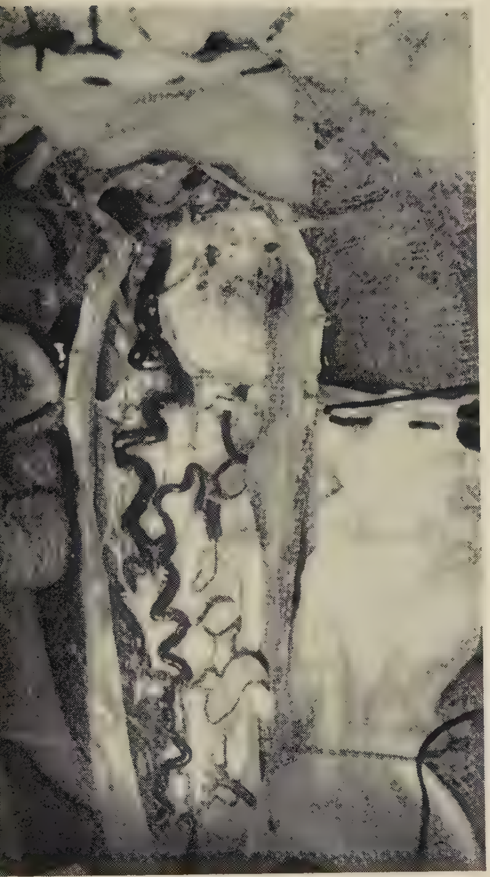

Fig. 1

Fig. 2

ROGERS AND PAYNE—THE DURA MATER AT THE CRANIO-VERTEBRAL JUNCTION

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Fig. 3

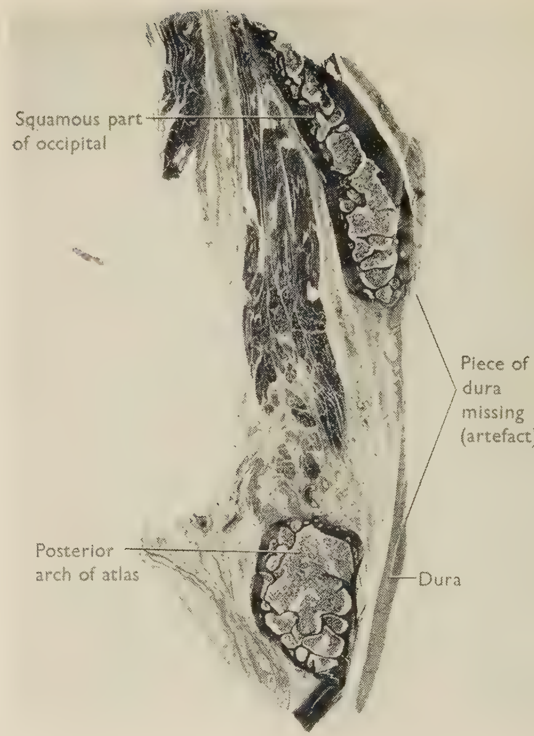

Fig. 4

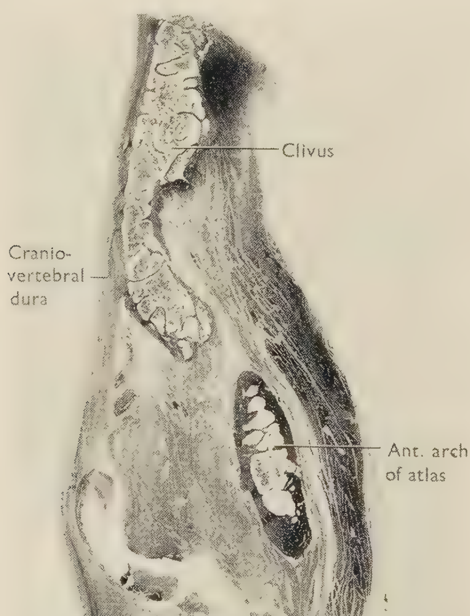

Fig. 5

ROGERS and PAYNE—The dura mater at the cranio-vertebral junction

THE THORACO-LUMBAR MORTICE JOINT IN WEST AFRICANS

By P. R. DAVIS

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INTRODUCTION

The thoraco-lumbar joint has been described by Topinard (1877) and Le Double (1912). At this joint the metapophyses of the lower vertebra are so formed that they help to transform the zygapophyseal joint into a mortice, which receives as its tenon the inferior articular processes of the vertebra above. A study of a considerable series of vertebral columns showed that the mortice is a normal anatomical feature in Europeans, occurring most frequently between the eleventh and twelfth thoracic vertebrae: clinical and experimental evidence suggested strongly that it marks the transition from thoracic modes of movement above the mortice to lumbar modes of movement below, and that, having relatively less mobility than its neighbours, it decreases the liability of the transitional vertebrae to suffer lesions from excessive torsional stresses, while it increases their liability to crush fractures (Davis, 1955, 1958). Insufficient data are available to assess any differences in mortice form in the two sexes, and little information is available regarding any racial differences in its occurrence and the time-sequence and manner of its development.

The present work presents the results of a study of the thoraco-lumbar mortice joint in a series of West African adult and juvenile columns of both sexes.

MATERIALS AND METHODS

The adult series, of macerated and dried material only, included forty-eight male and twenty-seven female vertebral columns from Yoruba and Ibo tribesmen in Nigeria. Both Yoruba and Ibo embrace a number of smaller tribes, whose separate identities are becoming lost through intermarriage.

The juvenile series, from the same two great tribes, included forty-three male and thirty-six female columns, of which thirty-three male and thirty-one female were macerated, cleaned and preserved complete with cartilage in formaline solution,

Table 1. *Stated age distribution of wet and dry preparations of different sexes in the juvenile series of West African vertebral columns*

Stated age (years)	Male		Female	
	Wet	Dry	Wet	Dry
0–2	12	—	10	—
3–5	16	—	15	—
6–8	4	1	4	—
9–11	—	2	—	1
12–14	1	4	1	2
15–17	—	3	1	2
Total	33	10	31	5

the remainder being macerated and dried preparations. The stated age distribution of these juvenile specimens is shown in Table 1.

It should be noted that, because of the paucity of written records, few West Africans know the true age of themselves or of their older children; however, the epiphysial ages of specimens in the present series were such that the stated ages appeared to be approximately correct.

No specimens showing pathological changes are included in either adult or juvenile series.

Each column was examined, and the level of occurrence of the mortice was noted in those possessing it. The depth of each mortice was classified as in previous investigations, a type I mortice completely enclosing its tenon, a type II enclosing more than half and a type III enclosing less than half (Davis, 1955).

RESULTS

(i) *Adults*

Forty-four of the forty-eight male columns possessed thoraco-lumbar mortice joints; of these forty-four, forty-three had a single mortice joint, and one had two mortice joints between three consecutive vertebrae. The remaining four columns had no mortice joint, the transitional vertebra having typical thoracic articular facets superiorly and typical lumbar articular facets inferiorly. Twenty-five of the twenty-seven adult female columns possessed a mortice joint, of which nineteen had a single mortice, and six had two mortice joints between three consecutive vertebrae. The remaining two columns had no mortice joint.

The levels of occurrence and depths of mortices in those with a single mortice joint are shown in Table 2.

Table 2. *Levels of occurrence and types of single thoraco-lumbar mortice joints in West African adults*

Level of occurrence	Male				Female			
	Type			Total	Type			Total
	I	II	III		I	II	III	
T10–11	—	—	1	1	—	1	—	1
T11–12	2	12	17	31	1	5	5	11
T12–L1	1	5	5	11	2	2	3	7
Total	3	17	23	43	3	8	8	19

The mortice joint occurs at different levels with a similar frequency in the two sexes; there is no significant difference when this is tested statistically ($\chi^2 = 1{\cdot}32$). The frequencies with which different depths of mortice occur in the two sexes are similar, there being no significant difference between them ($\chi^2 = 1{\cdot}42$).

The frequency of occurrence of columns with two serial mortice joints (1/44 male, 6/25 female) differs considerably, and this difference is significant (χ^2 with Yates's correction $= 6{\cdot}04$, $P < 0{\cdot}02$). In the female series with double mortices, five had mortices between T11–12 and T12–L1, the sixth having them between T10–11 and T11–12. The single male column had mortices between T11–12 and T12–L1. In

all six females the upper mortice was of type III, the lower mortice being of type I in five and type II in one. In the single male column the upper mortice was of type III, the lower being of type II.

(ii) *Juveniles*

In the juvenile series no columns with two mortices were seen in either sex. Table 3 gives the frequencies with which single mortices were found at different stated ages.

Table 3. *The frequency of occurrence at different ages of the thoraco-lumbar mortice joint in juvenile male and female West Africans*

Stated age (years)	Mortice joints: Males			Females		
	Absent	Present	Total	Absent	Present	Total
0–2	12	—	12	10	—	10
3–5	16	—	16	15	—	15
6–8	3	2	5	2	2	4
9–11	—	2	2	—	1	1
12–14	1	3	4	1	2	3
15–17	1	3	4	1	2	3
Total	33	10	43	29	7	36

The first appearance in the 6- to 8-year-old group suggests strongly that this is the age group in which the thoraco-lumbar mortice is formed.

The levels at which mortice joints occurred in the juvenile series are as follows:

	Males	Females
T11–12	7	5
T12–L1	3	2

These frequencies do not differ significantly when the sexes are compared ($\chi^2 = 0{\cdot}37$), nor do they differ significantly from the findings in the adult series ($\chi^2(♂+♀) = 0{\cdot}06$).

The depths of the mortices in the two sexes were:

	I	II	III
Males	—	8	2
Females	2	3	2

and again, in spite of the apparent paucity of group III mortices, these relative frequencies do not differ significantly from those found in the adult series ($\chi^2 = 3{\cdot}90$, $P > 0{\cdot}1$).

DISCUSSION

Out of a total of seventy-five adult West African columns of both sexes, sixty-nine were found to possess one or more thoraco-lumbar mortice joints, a proportion only slightly less than that of 98/102 found in Europeans by Davis (1958); this strengthens the hypothesis that the mortice must be regarded as a normal anatomical feature of the adult vertebral column.

The frequencies of occurrence of single mortice joints at different levels and of different depths were similar in both sexes in the adult and juvenile series, and again closely resembled the findings in Europeans. In contrast, the significantly high

proportion of female adult West African columns having two mortice joints shows a clear sexual dimorphic trend in those tribes, and also suggests that there are racial differences since only 1 out of 102 columns possessed a double mortice in Davis's European series of both sexes.

Nigeria is basically an agricultural country in which mechanical aids were rare until very recently. Thus most loads are still carried without benefit of the wheel. Whilst some Yoruba males may carry loads today, it is generally true to say that the West African female is made responsible for transport of burdens, as she has been for a very long time. The weights of such burdens vary enormously, but it is not an uncommon sight to see a young girl carrying a 10 gal. drum nearly full of water on her head, a burden weighing nearly 100 lb. The West Africans are on the whole of lighter build than their European counterparts (Roberts, 1960) so that such a burden is a very large load by English standards. It must therefore place relatively large stresses upon the trunk, including the vertebral column; it would therefore seem likely that reduplication of the mortice joint may well be directly associated with such habitual load-carrying.

This apparent association raises the question as to whether the mortice arises as a direct result of mechanical stress in the individual, or whether it arises as a genetically related characteristic. Were the mortice to appear in response to immediate stresses in a given individual, then one would expect it to be shallow at first and to become deeper later. That this is not so is revealed by the similarities in frequency with which each depth occurred in both the juvenile and the adult series. Thus heredity would seem to play a part in the development of these mortice joints, reaction to stress taking place only in those who carry the necessary hereditary factors, just as darkening of the skin in response to sunlight cannot take place except in those who possess the necessary allelomorphs.

Clearly the mortice tends to appear at 6–8 years in both sexes in West Africans. This is somewhat later than was suggested by a small European series (Davis, 1958), in which mortices were found as early as 3 years of age, but in both cases the mortice has appeared before one would expect the spine to be subjected to relatively large forces.

Comparison of Tables 1 and 3 might suggest that the mortice is only visible in dry preparations, but this possibility can be dismissed as in fact all those of both sexes in the 6- to 8-year-old groups possessing a mortice were wet preparations, as was one of the 12- to 14-year-old female columns.

CONCLUSIONS

It is concluded that the mortice is a normal feature of West Africans as well as of Europeans, and that it is a feature of post-natal development. Although the number of specimens is small, the findings suggest that the development of this type of intervertebral articulation is more likely to be under direct genetic control than to arise solely as a local response of the vertebrae to stress. The increased frequency of occurrence of a double mortice in West African females appears to be a racial feature and may be associated with their roles as habitual load carriers.

Gratitude is expressed to Prof. A. Smith of Ibadan for the use of his material, to Prof. Bowden for her helpful criticism and advice, and to the Royal Society and Nuffield Foundation for financial assistance.

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THE INNERVATION OF THE RENAL PORTAL VALVE OF THE DOMESTIC FOWL

By A. B. GILBERT

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One of the interesting features of the avian renal portal system is the valve at the junction of the renal and femoral veins. This valve was first described by Spanner (1925, 1939) and later by Sperber (1948); both have found it in all species of birds examined where it seems likely to be a fundamental feature of the portal system. Its influence on blood flow may be of importance to kidney circulation. This valve varies in shape from one species of bird to another and ranges from a thin membrane to a thickened funnel; the openings are one or more depending on the species, and on the type of valve (Spanner, 1925, 1939; Sperber, 1948). Spanner described it as being composed of epithelioid cells, connective tissue and smooth muscle tracts, Sperber stated that it has a great deal of muscle and Rennick & Gandia (1954) referred to it as the 'smooth muscle valve'.

Little is known about the mechanisms controlling the valve. Spanner's later paper (1939), which seems to have been generally overlooked, shows that histamine will act directly, causing the epithelioid cells to swell and the aperture of the valve to close; acetylcholine has the opposite effect. Rennick & Gandia (1954), on the other hand, have shown that although the valve is closed by histamine, it is also closed by acetylcholine and opened by epinephrine. It would therefore seem likely that this valve is under autonomic nervous control (Rennick & Gandia, 1954). This paper reports an investigation of the innervation of the valve.

MATERIALS AND METHODS

Brown Leghorns in the Poultry Research Centre flock were used. Birds of both sexes and between the ages of 12–24 weeks were killed by an overdose of Nembutal given intravenously.

For general histology the valve and the anastomosis (Text-fig. 1), together with the surrounding kidney tissue, were removed, embedded in paraffin and serially sectioned in the normal manner. Alternate sections were stained by haematoxylin and eosin and by Mallory's trichrome stain.

The distribution of the nerves within the valve was obtained by staining by one of the following methods, and mounting the valve entire.

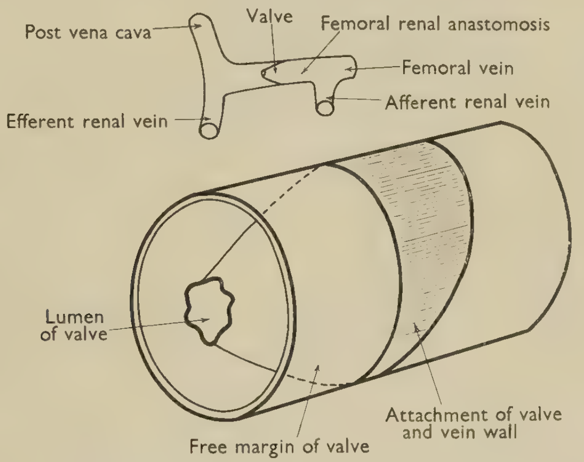
(1) *Mitchell's modification* (1953) *of Schabadasch's method* (1935)

This method is dependent on the affinity of living nervous tissue for methylene blue. Greater clarity is obtained by using both intra- and supra-vital techniques, and then differentiating with a suitable phosphate buffer.

5–10 ml. of a 2·5 % solution of methylene blue was injected into a wing vein using

a constant rate infusion pump during 30 min., the volume injected being dependent on the estimated blood volume of the birds (Newell & Shaffner, 1950). The birds were then killed and the valves, which were dissected out immediately, were stained for 15–20 min. in a 0·01 % aqueous solution of methylene blue at 37° C. Differentiation in the phosphate buffer was controlled under the microscope. At this stage the dissection was completed where necessary. Since it has been pointed out that the pH of the buffer and the 0·01 % methylene blue is critical (Meyling, 1953), pH ranges from 5·6 to 6·6 were tried and a pH of 6·2 gave the best results. The subsequent procedure was essentially the same as given by Mitchell.

Occasionally the valves failed to stain correctly, and the nerves were not easily identified. To control this variability small pieces of ureter, known to be innervated



Text-fig. 1. Diagram of the valve within the femoral-renal anastomosis. Inset: Diagram of the adjacent veins.

(Portman, 1950), were carried through the procedure together with the valve. In all cases where poor staining of the valve resulted the ureter was badly stained, and vice versa. It is difficult to account for this variability in some birds since at all times the solutions and techniques were identical.

(2) *The cholinesterase staining for nerve fibres of Coupland & Holmes* (1957)

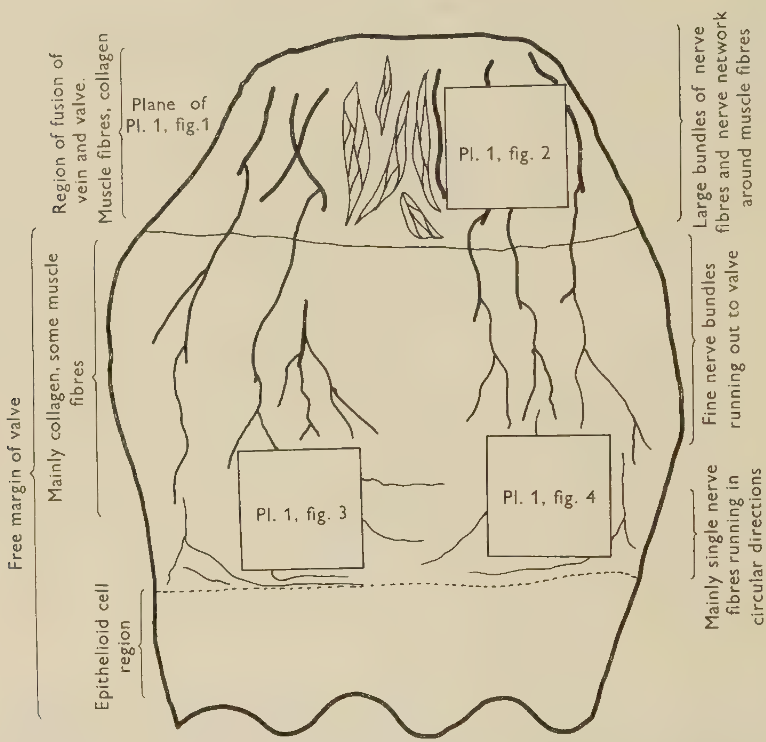
The entire valves, and in some instances frozen sections, were stained for cholinesterase. Controls were obtained by pretreatment with 1×10^{-7} aqueous solution of di-isopropylfluorophosphonate (DFP) (Gerebetzoff, 1959).

(3) *The Gros Schultze method* (*Cullins*, 1957)

(4) *The modified gold method of Ranvier* (*Garven*, 1925)

RESULTS

The apical third of the conical valve is composed almost entirely of epithelioid cells interspersed with collagen fibres and towards the base of the valve, where it fuses with the wall of the vein, muscle fibres become prominent. There is a gradual transition in the distribution of muscle fibres between these two regions. In the lower two-thirds of the valve the muscle fibres tend to form both longitudinal and circular tracts.



Text-fig. 2. Diagram of the distribution of the nerve fibres in the valve. The valve has been opened along the ventral surface and mounted flat. The general areas from which the subsequent figures were taken have been indicated.

With each of the stains it is abundantly clear that this valve is richly supplied with nerve fibres. Moreover, their distribution is not random, but falls into a recognizable pattern (Text-fig. 2). At the attachment of the valve and the vein relatively large bundles of nerve fibres can be seen (Pl. 1). Branches from these pass to the muscle fibres in the wall of the vein and valve junction, and terminate in a dense network, the meshes of which are elongated in the direction of the long axis of the smooth muscle cells (Pl. 1). Single nerve fibres also pass out from this network towards the valve apex. However, the main nerve supply to the free margin of the valve appears to originate as groups of fibres which divide from the larger bundles at the base of the valve. These groups run longitudinally just below

the periphery of the valve wall with repeated branching into single fibres ramifying in all directions. Near to the apex of the valve the remaining fibres fan out (Pl. 1, fig. 3). Before reaching the epithelioid cell region the course of the single fibres changes to a circular direction (Pl. 1, fig. 4); at no time have any nerve fibres been seen to enter the epithelioid cell region.

DISCUSSION

It has been clearly demonstrated that the valve in the renal portal system of the domestic fowl has a rich supply of nerves. These nerves are probably motor in origin since they appear to be closely associated with the nerves supplying the muscles at the base of the valve. Moreover, in the valve itself, the nerves always seem to terminate in the region of the smooth muscle cells, although it has not been possible to reproduce this photographically. It is extremely difficult to determine whether this autonomic nerve supply is sympathetic or parasympathetic at this level, although on the evidence of Rennick & Gandia (1954) it seems more than likely that both systems are present. There was no evidence of any sensory nerve endings with the methods used.

In view of the large numbers of nerve fibres it must be concluded that these are of importance in the control of the valve. This view does not invalidate Spanner's (1939) findings, and the control of the valve may also be affected without autonomic discharge.

The function of this valve still remains obscure. It will certainly regulate blood flow either to the kidney or to the heart depending on whether it remains closed or open, and Sturkie (1954) has suggested that the pressure relations within the various adjoining vessels may be of importance in this respect. So far the emphasis has been placed on the regulation of the blood to the kidney (Sperber, 1948; Rennick & Gandia, 1954). Rennick & Gandia suggested that the reason for opening of the valve in response to sympathetic discharge is to reduce the load on the kidney when muscular activity of the legs is high. On the other hand, since the avian renal portal system has been shown to be functional (Sperber, 1948; Rennick & Gandia, 1954; Siller & Carr, 1961; Sykes, 1960*a*, *b*; and others), it may be that the valve acts not so much as a 'safety device' when blood flow is high, but rather to ensure an adequate supply to the kidney when the blood flow from the legs is low (Sperber, 1948).

One other possibility suggested by the word 'Drosselklappen' used by Spanner (1939) is that this may be of more importance in regulating the flow of blood to the heart, and hence cardiac output; the flow to the kidneys being more or less a shunt for blood not required by the heart. In support of this Rennick (personal communication) has observed that in excitement, when the cardiac output is increased, the valve remains open.

However, the lack of information regarding flow and pressure relationships within the larger vessels of this region makes it difficult to assess the functional significance of this unique valve, but in view of its definite structure and innervation it seems clear that it is of importance to the avian circulatory system.

SUMMARY

1. The innervation of the renal portal valve of the domestic fowl was investigated.
2. A rich supply of autonomic nerves was demonstrated by all methods used.
3. These nerves are possibly motor in origin; no evidence was obtained that sensory fibres were present.

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EXPLANATION OF PLATE

Fig. 1. Transverse section of the junction between the valve and vein wall. Large bundles of nerve fibres can be seen together with some groups of fibres of the nerve network. Cholinesterase preparation. Frozen section. ×95.

Fig. 2. Portion of entire valve showing dense nerve network in muscle fibres at the junction of the valve and vein wall. Cholinesterase preparation. ×320.

Fig. 3. Fine terminal nerve fibres near the apex of valve. These are initially collected into one bundle and are seen beginning to fan out. Methylene blue. Entire valve. ×650.

Fig. 4. Terminal branching nerve fibre, which has started to change direction to a circular course. Methylene blue. Entire valve. ×750.

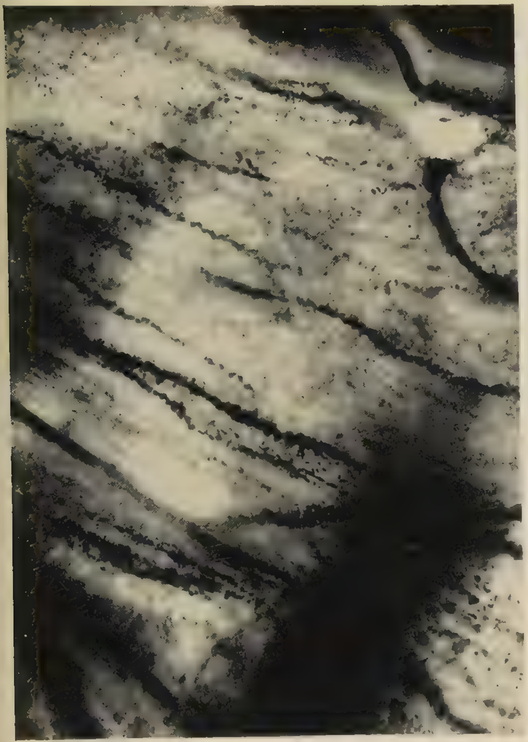

Fig. 2

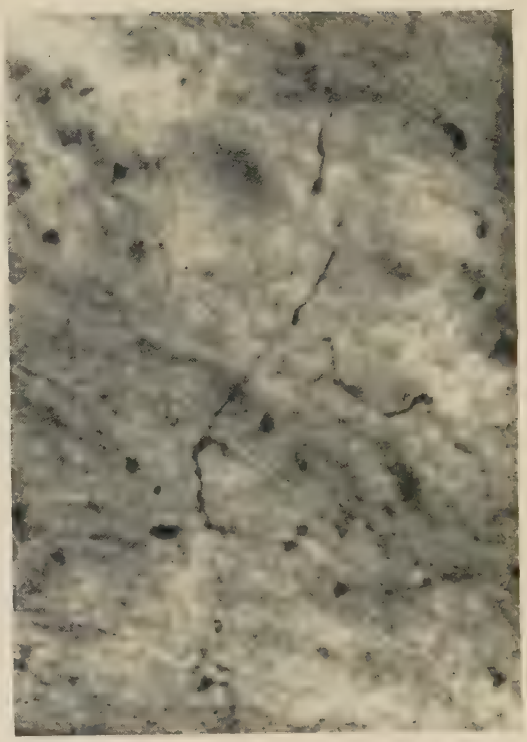

Fig. 4

Fig. 1

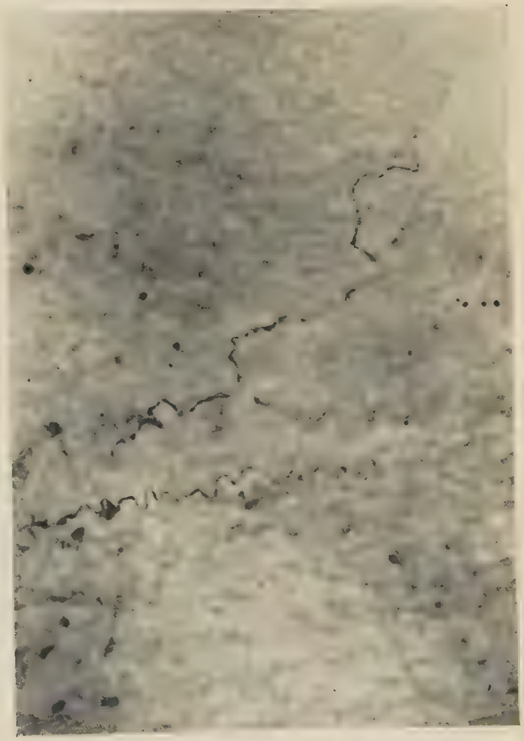

Fig. 3

GILBERT—INNERVATION OF THE PORTAL VALVE

(*Facing p.* 598)

REVIEWS

Synovial Joints. Their Structure and Mechanics. By C. H. Barnett, D. V. Davies and M. A. MacConaill. (Pp. 304; text-figures 60. 50*s.*) London: Longmans, Green and Co. Ltd. 1961.

One of the obvious deficiencies in anatomical literature has been a critical up-to-date survey of the articular tissues along with the factors that are concerned in the mechanism of synovial joints. It is most appropriate that three senior anatomists, who have all long attained an international reputation on different aspects of synovial joint structure and function, should have now collaborated in the production of this intellectually stimulating monograph. The structure of individual joints and the movements at them are adequately considered in most standard text-books, but usually the story ends there except for brief, and frequently inadequate, notes on the main features of joint tissues.

This text is divided into four sections and each of these has several chapters which, for the most part, are complete in themselves. In Section 1 there is a fairly detailed account of cartilage, synovial membrane, the fibrous capsule and ligaments and the intra-articular structures, with a separate chapter devoted to each of them. Each tissue is considered at all levels of investigation, ranging from the naked-eye to the electron microscopic and there is a full account of the chemical composition of synovial fluid. Section 2, which is entitled 'The Biology of Joints', is concerned mainly with the nutrition and respiration of joint tissues and their growth and degeneration. Section 3 commences with a short chapter on the anatomical classification of joints. The classification of joints is a vexed question and it is not necessary to read between the lines to assess the author's opinion on some of the inadequacies of the *Nomina Anatomica* (1955, 1960). The reviewer agrees with the authors that a satisfactory nomenclature should be indicative of function as well as structure. Most of this third section is devoted to the mechanics of joints, and although this mathematical aspect of anatomy is approached in a simple manner it is obvious that the writer is not only a master of the geometry of articular surfaces but is also an anatomical teacher with a selective aptitude for leading from the simple to the complex. Section 4 is the shortest of the four sections but it contains a wealth of basic facts, some long established and others recently described, on joint movements and their co-ordination.

These might justifiably be regarded as some of the principal features of this book, but such an enumeration of its contents gives no indication of the value that this new work will have for anatomists, clinicians and for those whose life work is largely concerned with the treatment of joints. The 500 references constitute a selective bibliography and, as nearly half of them refer to papers of the last decade, they constitute an invaluable guide to the research worker embarking on a problem on joints.

The figures are for the most part excellent and the fact that most of the microphotographs are of human tissue should appeal to anatomist and clinician alike. The blood supply and nutrition of articular tissues are all-important, but there would appear to be a lack of balance in having some ten photographs of injected vessels, informative and beautiful as they are, and none illustrating the nerves of the capsule; it would seem unfortunate to have such an excellent and critical survey of the present-day knowledge of the nerve supply of joints completely unillustrated.

This is an inspired work and it is obviously written by workers who are dedicated to the study of Arthrology. Its text is refreshing, lucid and critical and there is a subtle blending of what is new and what is long established. The authors are to be congratulated on this notable contribution to medical literature and there can be little doubt that it will soon pass into a second edition.

To have dedicated the book to Thomas Walmsley is in itself an act of thoughtfulness and grace and the reviewer knows well that he would have been exceedingly proud to see this monograph emanating from a British Press.

ROBERT WALMSLEY

Spalteholz–Spanner Handatlas der Anatomie des Menschen. Edited by R. SPANNER. (Vol. 1, pp. xvi + 420; 701 figures. Vol. 2, pp. xii + 531; 929 figures.) Sixteenth edition. Scheltema and Holkema, Amsterdam. 1959–61.

This is the sixteenth edition of Spalteholz's atlas which has occupied a prominent place amongst anatomical books for over 60 years. It is amongst the most comprehensive of modern anatomical atlases. After a brief glossary of terms and a table of the segmental innervation of the muscles, there is a general section devoted principally to the detailed structure of bone. This is followed by the special anatomy, which occupies the major part of the two volumes and is arranged on a systematic basis with a clear if brief description of the structures depicted in each figure. All the figures are clearly drawn and fully labelled. The majority are of dissections, injections and osteological specimens but radiographs, histological sections and the electron micrographs are also included. Where necessary there are diagrams to explain the anatomical arrangements and, in appropriate plates, the development of the part or structure. There is a comprehensive index. Since the last edition many of the figures have been redrawn and new ones have been added. This atlas, which of later years has been seen only infrequently in this country, still maintains its high standard of accuracy and can be wholeheartedly recommended to the student and teacher.

D. V. DAVIES

Essentials of Human Anatomy. By R. T. WOODBURNE. (Pp. vii + 620; 403 figures; 100*s*.) Second edition. Oxford University Press. 1961.

The general arrangement of this volume remains as in the first edition. The principal changes include the improvement of several figures and the inclusion of new ones. The latter include ten full colour plates 'illustrating central anatomical features in each region of the body'. These plates are beautifully produced; they would, however, be more useful if they had been numbered and received reference in the appropriate place in the text.

Avoiding much detail, particularly detailed relationships, this book is suitable for beginners. According to our present standards, however, it is barely adequate as the sole text on topography for the 'second medical examination' candidate. There is some elementary histology and, here and there, the author makes excursions into embryology and physiology. References to a selection of articles and books are found at the end of each chapter. In the case of some of the books cited it is a pity that the author does not instance the latest edition. Thus the 1945 edition of *Tissues of the Body* and the fourth edition of *Frazer's Anatomy of the Human Skeleton* are listed.

The text is, as far as it goes, accurate and there is a good index. The whole volume is well produced on good quality paper and in clear type. The terminology followed is in the main the P.N.A. Some radiographs are included but several of these are too small to show details of structure, particularly of the bone.

The book will help not only the beginner but also those who find it difficult to select the significant matter from larger and more detailed texts.

D. V. DAVIES

BOOKS RECEIVED

Beiträge zur vergleichen Morphologie des Nagetiergehirnes. By G. PILLERI. *Acta Anatomica*, Vols. 39 and 42 (Suppls. 30 and 40), 1961. (Pp. 1–84, 49 figs., SFr. 15.) S. Karger, Basel.

Sixteenth Annual Report of the Nuffield Foundation. Oxford University Press, 1961.

PROCEEDINGS OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

APRIL 1961

An Ordinary Meeting of the Society for the Session 1960–61 was held on Wednesday, 26 April 1961, in the Department of Anatomy, Royal Free Hospital School of Medicine, London, W.C. 1.

The President (Prof. J. D. Boyd), Prof. J. J. Pritchard and Prof. R. J. Harrison occupied the Chair at the various sessions.

The following are the authors' abstracts of papers presented.

The arterial blood supply of the lumbar and sacral nerve plexuses in the human foetus. By M. H. DAY. *The Royal Free Hospital School of Medicine, London*

Previous work on the blood supply of this region usually forms part of general descriptions of the blood supply of roots, ganglia and peripheral nerves, or of general works on the distribution of arteries. Later workers have tended to restrict their attentions to the nerves of the lower extremity and in particular to the sciatic nerve alone.

Observations were made on thirty lumbar and sacral plexuses of human foetal material, injected with neoprene latex, to determine the gross arterial blood supply of this region. The injections were performed using manometrically controlled pressure. Subsequently twelve specimens were dissected and the remainder were cleared in tetrahydro-naphthalene before the observations were made.

The results showed that these nerve plexuses obtained their blood supply principally from the lumbar spinal arteries (segments L_1–L_5), and the lateral sacral arteries (segments S_1–S_4). Other vessels contributing to the blood supply of these structures included the lumbar arteries, the superior and inferior gluteal arteries and the ilio-lumbar artery.

The intra-neural vascular arrangements were studied by the use of Pickworth's method and MacConaill's FALG techniques.

The results obtained appeared to confirm what is already known of the blood supply of the roots, ganglia and nerves forming these plexuses.

Further observations on the innervation of avian muscles. By S. H. SHEHATA and RUTH E. M. BOWDEN. *Royal Free Hospital School of Medicine, London*

In a previous communication (Shehata, S. H. & Bowden, Ruth E. M., *J. Anat., Lond.*, 1960, **94**), it was shown that some muscles in the chick had diffusely scattered motor end-plates, whilst in others they were confined to localized areas. The fibre size has been studied in the nerves of each muscle investigated. The histograms showed a unimodal fibre size distribution in every case, but two ranges of fibre sizes have been found. In one, the nerves ranged from 2 to 14 μ in diameter, with the peak between 6 and 8 μ. This was associated with the diffuse type of motor end-plate distribution except in one case. In the second type, the diameter of nerve fibres ranged from 2 to 20 μ with the peak between 8 and 14 μ and in each case this was associated with a zonal distribution of motor end-plates. Frozen transverse sections of these muscles stained with Sudan 3, H. & E., and for succinic dehydrogenase were studied and correlated with the previous data.

Studies on the growth of skeletal muscle fibres.
By B. Mackay. *University of Edinburgh*

Crawford (*J. Bone Jt Surg.* 1954, **36** B) studied the growth in length of the rabbit tibialis anterior by implanting tantalum wire markers in the muscle belly and following their changing positions by X-raying at intervals over a period of growth. As Crawford pointed out, a difficulty in interpreting the results from such an experiment was the lack of knowledge of the arrangement within the muscle of its component muscle fibres. The muscles of the rat were found to possess relatively simple arrangements of their muscle fibres and, in the present studies, growth of these muscles was studied with tantalum wire markers.

Up to four markers were implanted in various muscles of 3-week-old rats, and their positions followed over a period of about 3 months. The markers were found to separate evenly from one another, maintaining their positions, relative to one another and to the end of the muscle. The results agree with those obtained by Crawford.

These findings indicated that interstitial growth was occurring, but it seemed likely that the movement of the wire markers was due to growth of the connective tissue of the muscle rather than to growth of the contractile substance itself.

Aspects of the ecology of African primates. By J. R. Napier. *Royal Free Hospital School of Medicine, London*

The existence of stratification in the structure of tropical rain forests has been a matter of dispute for many years. The technique of constructing profile diagrams of selected forest areas which was developed by Davis & Richards (*J. Ecol.* 1933, **21**) in Guiana has largely settled the controversy.

Since then this method of study has been applied by Richards and others to African rain-forests in Nigeria, Uganda and Ghana and in other parts of the world. Profile techniques have shown the existence of three strata in tropical rain-forests, namely: an upper storey (or emergent layer), a middle storey and an understorey, the latter invariably forming a closed canopy.

Stratification studies, apart from their significance to botanists and foresters, have already provided valuable information for virologists in relation to the epidemiology of yellow fever. Little information is as yet available on the ecology of primates in terms of forest levels, but the importance of this aspect of primatology is now becoming recognized (Booth, A. H., *J. W. Afr. Sci. Ass.* 1956, **2**). It is hoped that further field work of this nature will provide valuable data for taxonomists as well as for those interested in the phylogeny of the primates particularly in respect of their locomotor patterns.

The demonstration is an attempt to correlate the ecology of vegetation with that of the primates in terms of (1) vegetational zones, (2) stratification levels in tropical rain-forests. The findings are based on information from many sources.

Autonomic nerve supply and distribution of cholinesterase activity in glands and unstriped musculature. By N. Cauna and N. T. Naik. *King's College, Newcastle upon Tyne*

Neurohistological and histochemical studies have been carried out on human and mammalian tissues using a modified Bielschowsky–Gross silver method and Koelle's technique for demonstrating cholinesterase activity.

It has been found that the somatic nerve endings are anatomically defined and are associated with specialized cellular elements by which they can be recognized. Histochemically, a variety of somatic end-organs give a positive cholinesterase reaction, localized in the nerve ending or in the associated cells.

Autonomic nerves do not end in defined terminals or in a reticulum. Instead, the terminal segments of the postganglionic fibres proceed in close relationship to glandular tissues or unstriped musculature without ending separately on individual secreting or muscle cells. Histochemically, certain postganglionic fibres give a positive cholinesterase reaction especially in their terminal segments which apparently constitute their zone of transmission. In addition, tissues supplied by the autonomic fibres, or certain adventitial elements related to such tissues, usually give a positive cholinesterase reaction.

It is suggested that the presence of cholinesterase in muscle fibres or glandular cells, or in the adventitia surrounding the acini, may promote the spread of a stimulus without the need for a direct supply of each individual cell by a nerve fibre.

Transneuronal cell degeneration in the olfactory bulb of the rabbit. By M. R. Matthews and T. P. S. Powell. *University of Oxford*

A study has been made of transneuronal cell degeneration in the main and accessory olfactory bulbs of the rabbit at periods varying from 6 to 200 days after destruction of the olfactory mucosa. All layers of the bulb, except the periventricular layer, show severe shrinkage, and the periglomerular, tufted, mitral and granule cells undergo transneuronal atrophy. Sections stained with Bodian's protargol method show that the dendrites of the mitral and tufted cells atrophy and that there is loss of fine fibres in the outer part of the molecular layer subjacent to the lateral olfactory tract. All these changes begin before the end of one month after denervation. Golgi–Cox preparations show that periglomerular, tufted and mitral cells which are undergoing transneuronal degeneration are more resistant to impregnation by this method. An incidental finding in these experiments is the absence of involvement of the epithelium of the vomero-nasal organ in atrophic rhinitis.

Daily variations in numbers of active palmar sweat glands. By Jean Harrison. *Royal Free Hospital School of Medicine, London*

Active sweat glands in a standard area of palmar skin were counted daily under resting conditions in twenty healthy young men.

The subjects appeared to fall into one or other of two clearly distinct groups, those whose sweat counts were regular from day to day (mean variance 40·2), and those whose counts were very irregular (mean variance 685·8). Whilst the average values were significantly different between individual subjects, the difference between the over-all average values of the two groups was highly significant.

In order to test the consistency of these two patterns of behaviour of the palmar sweat glands a second similar investigation was made some months later on six members of each group. Although the average values had altered with the passage of time in some individuals, the pattern of regular, or irregular, sweating remained unaltered in every case.

Other physical and psychological properties which might also tend to differentiate these two groups of subjects are being investigated.

Langerhans cells in human sebaceous glands. By A. S. Breathnach and J. Everall. *St Mary's Hospital Medical School, London*

Gold-positive dendritic cells (Langerhans cells) can be seen in sections of human sebaceous glands stained by Gairn's gold chloride technique. These cells are present throughout the length of the excretory ducts and also in that portion of the acinar wall which is directly continuous with the duct. An occasional gold-positive cell may be encountered in the depth of an acinus within an epithelial septum separating two locules.

The Langerhans cells of the surface epidermis are commonly thought to represent effete melanocytes, but some workers regard them as 'neuro-hormonal elements', or as structures closely related functionally to the superficial nerve supply to the skin. The bearing of the present findings on these conflicting views was discussed.

Continuous measurement of linear diameters of the chest. By P. R. Davis. *Royal Free Hospital School of Medicine, London*

There have been many observations on movements of the chest during respiration; less has been written about the effect on the chest wall of activities of the arms. No observations appear to have been made of the effect on chest diameter of lifting weights: there are mechanical difficulties in recording such effects, and a continuously recording caliper has been designed which gives a linear response for movements of up to 4 cm.

Recordings from eight male subjects reveal considerable variation in the degree of movement at rest in both erect and stooping positions. In the erect position lifting a weight has but little effect on the chest wall. Lifting a weight in the stooping position decreases the amplitudes of chest movement at both upper and lower levels. If the weight is heavy enough to induce a *lift* pressure within the trunk, then the chest is held rigid for prolonged periods between breaths, and may be held at a narrower diameter with each successive breath. When an initial *snatch* pressure occurs, the chest widens as the pressure increases, suggesting that snatch pressures are caused mainly by ascent of the diaphragm. Simultaneous measurements of chest diameters and intrathoracic pressures show that snatch pressures cause slight oscillation of the chest wall.

Atresia of the vagina: an anatomical, embryological and endocrinological analysis. By P. Bacsich and W. M. Dennison. *University of Glasgow*

Atresia of the vagina in the newborn, leading to the development of hydrocolpos or pyocolpos is a relatively rare congenital anomaly. In fact, even if we include the five cases observed by us, there are reports of less than fifty cases in the medical literature of the last one hundred years.

Symptomless atresia of the vagina in the adult is, on the other hand, far more commonly encountered.

The main feature of both groups of cases is the atresia of the vagina and one can legitimately ask why there is such a marked difference in the clinical behaviour.

Study of the anatomical conditions in the two groups of cases reveals that in neonates the abnormality is confined solely and exclusively to the vagina, while in the adults, apart from the atresia of the vagina, usually there is gross hypoplasia or aplasia of the uterus and the tubes, coupled, occasionally, with agenesis of the ovaries.

From the embryological point of view, in neonates the anomaly is strictly confined to the derivatives of the urogenital sinus (sino-vaginal bulbs), while in the adult cases organs of Müllerian origin are also involved.

Recent studies indicate that the secretogenic stimulus for the cervical glands of the uterus is provided by oestrogens. The relatively high concentration of oestrogens in the blood of the neonatal female, derived from the placenta and the foetal ovaries, results in a prolific mucous discharge from the cervical glands. It is the accumulation of this mucous secretion which leads to the development of hydrometrocolpos in the cases of vaginal atresia of the first group, while in the second group, owing to the hypoplastic or aplastic condition of the uteri, no appreciable quantities of secretion can be produced and no mucous stasis develops.

On the basis of the above considerations we believe that the two groups of atresia of the vagina should be treated as separate clinical entities.

Insulin and the early chick embryo. By Patricia Barron. *University of Aberdeen*

In a previous communication to the Society (July, 1960), it was reported that application of varying concentrations of therapeutic insulin to early chick embryos, explanted by the New technique and cultured *in vitro*, resulted in marked inhibition of the development of brain and neural tube and slight inhibition of somite formation, but in only a very few cases did it interfere with the development of the heart.

Further experiments have been carried out using intermediates, in the carbohydrate metabolic chain as supplements to insulin treatment, in order to determine where and how the insulin is interfering in the pathway. Sodium pyruvate, nicotinamide and diphosphopyridine nucleotide have been applied with insulin to the dorsal and ventral surfaces of the embryo.

The results of these experiments were reported and their significance discussed.

The origin of the hepatic haemocytoblasts. By D. Brynmor Thomas and J. M. Yoffey. *University of Bristol*

While there is clear histological evidence of active erythropoiesis in the liver of the human foetus and red cell-precursors are abundant in the hepatic trabeculae, the mesenchyme around the intra-hepatic vessels contains very few haemopoietic cells and is composed of an essentially homogeneous population. There are no cells in the foetal liver which are transitional in form between the typical mesenchymal cell and the numerous haemocytoblasts with which the hepatic trabeculae are infiltrated. The trabeculae however contain numerous cells intermediate in form between haemocytoblasts and the cells of entodermal origin, so that a complete morphological spectrum linking the two can be constructed readily. Thus the undifferentiated cells of the entodermal component appear to be the haemopoietic stem cells in the human foetal liver. These cells have round or ovoid, vesicular nuclei and diffuse, pale cytoplasm. The sequence of changes whereby they give rise to haemocytoblasts appears to commence with the development of cytoplasmic basophilia. As the cytoplasm becomes progressively more basophilic it condenses to form a narrow shell around the nucleus and the cell assumes a spherical or oblate shape. The nuclear chromatin becomes more dense and the cell, after separating from those around it displays the features characteristic of a typical hepatic haemocytoblast. While most of the haemocytoblasts appear to differentiate *in situ*, some are found lying free in the hepatic sinusoids, whence they may be carried in the blood to differentiate elsewhere.

The placenta of the Ca'ing whale, *Globicephala melaena* (Traill). By W. R. M. Morton and H. C. Mulholland. *Queen's University, Belfast*

The pregnant uteri of several specimens of Ca'ing whale, or Black Fish, caught in the Faeroes in July 1960, were examined by one of us (H.C.M.) shortly after the animals had been killed. The placenta was diffuse in each case. Portions of a uterine wall with attached foetal membranes were removed and fixed in 10 % formol-saline. Microscopical examination shows that the chorion is covered with small villous tufts about 2 or 3 mm. high which fit into corresponding crypts in the uterine wall. These crypts appear as diamond-shaped depressions running longitudinally along the shallow folds of the uterine epithelial lining. Coiled uterine glands occupy much of the wide subepithelial zone and appear to open at the base of certain of the maternal crypts. The maternal/foetal relationship of the placenta can be described as 'epithelio-chorial', although the maternal capillaries in many crypt walls are intra-epithelial in position. The foetal blood vessels lie below the thin trophoblast and close to the maternal vessels. The cytotrophoblast cells opposite coagulated uterine gland secretion are columnar or cuboidal in shape, with frothy cytoplasm or deeply staining firm cytoplasm. These cells appear to be absorptive in nature. The chorion is lined by a partially detached membrane, the free surface of which is covered by a low cuboidal epithelium. This membrane is probably the wall of the allantois.

Experimental production of 'Swan necks' in rat nephrons. By Julia Fourman. *University College, Cardiff*

Previously 'Swan necks' have been reported in Franconi's syndrome and in congenital nephrosis. When slices of kidney from a 15-month human infant dying from idiopathic hypercalcaemia were macerated in HCl and individual nephrons teased out they were again found. In many nephrons the proximal convoluted tubule was abnormally thin for the first part of its course, while the glomerulus retained a normal shape. Nephrons from eight stillborn premature human foetuses (1040–2210 g.) did not have these thin 'Swan necks'. They are not a stage in development of the human nephron nor are they present in the normal mammalian kidneys, although similar 'Swan necks' are present in amphibia.

In the case described the juxtamedullary nephrons were all normal, and the most peripheral nephrons had the longest 'Swan necks'. In man at birth only the juxtamedullary nephrons are mature. Thus the more peripheral developing proximal tubules would in this case of hypercalcaemia have had to deal with an increased filtration load of calcium, which may have produced the developmental abnormality.

To test this hypothesis eight neonatal rats were given, for 3 weeks, daily injections of calcium gluconate which has been shown to produce hypercalcaemia. 'Swan necks' were found in many nephrons from the kidneys of these rats, suggesting that these are the result of a biochemical abnormality acting on the developing nephron.

Observations on the aetiology of osteogenesis imperfecta in man and carnivores. By Patricia P. Scott. *Royal Free Hospital School of Medicine, London*

In human beings, fragility of the skeleton, due to failure of normal development of bone, is found in the condition known as osteogenesis imperfecta. Bones have narrow cortices and reduced radiological density; spontaneous fractures occur *in utero*, or result from minor traumatic incidents in childhood. Characteristically, there is marked improvement as the individual matures, bones increase in density and fractures occur less frequently, although final stature is reduced and deformities, due to previous fractures, persist. The condition has been shown to be the result of a congenital defect in bone formation, which is heritable and not directly related to the metabolism of calcium and phosphorus.

An osteodystrophy, having superficially similar signs to the human disease, has been noted in carnivores in many parts of the world, and attempts have been made to draw a parallel between the two conditions. Evidence was presented which suggested that the animal disease is of environmental origin rather than due to a genetic defect of this kind responsible for osteogenesis imperfecta.

Dye deposition in the skeleton and teeth of rats following intra-vital alizarinization. By C. H. Tonge. *King's College, Newcastle upon Tyne*

A single intra-peritoneal injection of 0·2 ml. of a 2 % sodium alizarin sulphonate (C.I. 1034) solution was administered to rats aged birth–14 days. Following injection the animals were sacrificed at intervals from 24 hr. to 7 days. Serial paraffin sections were made without previous decalcification and after fixation in 10 % neutral formalin. Various counterstains including haematoxylin, Weigert's elastic, silver, toluidine blue and methylene blue were applied to alternating sections in order to stain the tissues adjacent to the intra-vitally alizarin-stained bones and teeth. Alizarin-stained areas of active calcium deposition were demonstrable in the skull and appendicular skeleton. In the teeth the greatest dye concentration occurred in the central area of the enamel and between it and the surface and also at the junction of the predentine and dentine. In animals sacrificed 5 days after injection general staining of tissues is observed, although the areas stained at the time of injection are still recognized. There is some evidence that bone growth has

occurred in the epiphyseal regions since the injection, but no evidence suggesting subperiosteal bone deposition. Injection of pregnant rats fails to stain the foetuses.

A telemetering electromyograph for investigating muscles in walking. By J. Joseph and C. K. Battye. *Guy's Hospital Medical School, London*

There are two main problems in investigating the muscles used in walking by means of an ordinary electromyograph—either a treadmill has to be used or very long leads whose movement causes interference. The Electronics Workshop at Guy's Hospital Medical School has made a portable electromyograph and transmitter which enables the subject to walk normally at a considerable distance from a receiver which picks up the electric changes due to the activity of the muscles being investigated. This was demonstrated and preliminary results obtained from a study of the tibialis anterior and calf muscles presented. During the swinging phase the tibialis anterior shows no electric activity for a short period of $\frac{1}{10}$ sec. before showing marked activity just as the heel is placed on the ground at the stance phase.

Radiation, growth rate and the haemoglobin oxidation sensitivity of rats. By W. K. Metcalf. *University of Bristol*

The sensitivity of the haemoglobin of rats to oxidation by nitrites seems to be directly related to their growth rate. To obtain a better understanding of this phenomenon, attempts have been made to interfere with the growth rate by radiation to see if the haemoglobin-sensitivity is affected in the appropriate way.

500 Röentgen of gamma radiation were administered, at 18 r./min., to rats of various ages. The growth rate of suckling rats is hardly affected by this dose of radiation, and there is no immediate change in their haemoglobin-sensitivity although it does remain high for a prolonged period after radiation. Weaned and adolescent rats, however, show no growth for a few days following radiation, and their haemoglobin-sensitivity, after a short period of depression during this pause in growth, increases to that of infant rats for a prolonged period. Mature rats lose a little weight and their haemoglobin-sensitivity falls to a low level within 24 hr. and remains there for several weeks.

The long term increased sensitivity following 500 r. appears to be of quite a different nature to that in childhood, pregnancy, and following starvation, in that it is completely unaffected by the administration of riboflavine subcutaneously.

Changes in first regional lymph nodes draining second-set homografts and autografts of fresh cancellous bone. By R. G. Burwell. *University of Leeds*

The immune responses of lymph nodes draining first-set homografts and autografts of fresh cancellous bone have been reported (Burwell, R. G., *J. Anat., Lond.*, 1960, **94**). These findings have been used as a base-line to compare the responses of nodes draining homologous bone treated by several of the methods used to prepare bone for the bone bank (Burwell, R. G., *J. Anat., Lond.*, 1960, **94**).

The reaction of the first regional lymph node to a constant weight of fresh homologous cancellous bone inserted 3 weeks after the introduction of a similar graft from the same donor into the subcutaneous tissue of the same ear, has been investigated. Including the control studies of nodes draining second-set autografts, fifty-five adult male rabbits were used in the investigation.

The increase in weight of the first regional lymph nodes on the side receiving second-set homografts is more rapid and of greater magnitude than in animals receiving first-set homografts. Second-set autografts evoke weight changes similar to those in nodes draining first-set autografts.

The main histological differences between first-set and second-set responses in lymph nodes draining homografts of cancellous bone are: (*a*) the first appearance of large and medium lymphoid cells is earlier after second-set than after first-set homografts; (*b*) the distribution of large and medium lymphoid cells in the cortex is subcapsular in second-set and is throughout the cortex in first-set responses; (*c*) the germinal centres become larger and more numerous in nodes draining second-set grafts after the 5th day, whereas they tend to disappear in nodes draining first-set grafts between the 4th and the 8th day; (*d*) the medullary enlargement is earlier in second-set than in first-set responses.

These findings were discussed in the light of knowledge concerning the destruction of second-set homografts of normal tissues.

Mucopolysaccharides in inflammation. By R. Barer, G. R. Barer and S. Bradbury. *University of Oxford*

Although much effort has been devoted to the study of the localization of inflammatory processes, relatively little is known about the detailed mechanism. A common view is that a fibrin barrier is laid down between the normal and the inflamed tissues. An examination of inflammatory cells by electron microscopy suggested that mucoproteins or substances concerned with mucoprotein formation might occur in such material. Histochemical tests were carried out on sections from freshly produced turpentine abscesses in the ear of the rabbit and on a large number of blocks of turpentine and bacterial abscesses available from a previous investigation (Barer, G. R., *Brit. J. Exp. Path.* 1952, **33**) in all cases the appearance of a cellular exudate was accompanied by a very strong PAS reaction for neutral mucopolysaccharide. The polymorphs gave particularly strong reactions, often confined to granules. Material histologically identified as fibrin also gave a positive reaction. Glycogen was excluded by enzymic digestion. The PAS reaction was blocked by acetylation and the blockage could be reversed by treatment with KOH. Tests for acid mucopolysaccharides gave at most a very faint reaction except in the ear cartilage. However, after sulphation the inflamed region reacted very strongly as well. These facts support the view that considerable amounts of neutral mucopolysaccharides are present in and around the abscesses. These substances might be deposited on a framework of fibrin and in view of their high viscosity could play an important part in limiting the spread of an inflammatory process.

The origin of mesothelial cells during the repair of peritoneum. By F. R. Johnson and H. W. Whitting. *London Hospital Medical College, London*

The present investigation was undertaken to determine the origin of mesothelial cells participating in the repair of peritoneal lesions. Theories prevailing are: (1) that they arise by proliferation of surrounding undisturbed cells, (2) that they are seeded on to the site of the lesion from adjacent peritoneal surfaces, and (3) that they arise by metaplasia of fibroblasts, monocytes or undifferentiated mesenchymal cells.

Two areas of peritoneum, approximately 1 cm.^{2} in size, were removed from the anterior abdominal walls of thirty-three rabbits. In each animal one of the denuded areas was left bare and the other was covered with Polythene to prevent contact with the contents of the peritoneal cavity.

Within 24 hr. scattered mesothelial cells were found on the uncovered sites and on the exposed surface of the Polythene. These cells rapidly increased in number and eventually formed a continuous layer of cells. During the early stages of healing mesothelial cells were not seen deep to the Polythene. In the later stages of healing the mesothelial cells of the peritoneum peripheral to the lesions increased in size, became cuboidal and showed signs of proliferation followed by migratory activity.

These findings suggest that two processes are involved in the repair of peritoneum, namely, a rapid seeding of raw surfaces and a much slower proliferation and migration of mesothelial cells peripheral to the site of injury. No evidence of metaplasia of connective tissue elements was found in this study.

JUNE 1961

The Summer Meeting of the Society for the Session 1960–61 was held on Thursday and Friday, 29 and 30 June 1961, in the Anatomy Department, University of Glasgow.

The President (Prof. J. D. Boyd) occupied the Chair.

The following are the authors' abstracts of papers presented.

The fine structure of the epithelial cells of the colon.

By G. M. Wyburn. *University of Glasgow*

These are the columnar epithelial cells lining the lumen and the principal and mucous cells in the crypts of Lieberkühn. Features of special interest in the epithelial cells of the lumen are the microvilli, superficial vacuoles, terminal bars, and interdigitating cell membranes. In the cells lining the crypts the lateral bounding membranes separate a third of the way down the cell leaving well marked intercellular spaces broken up by projecting microvilli which, a short distance from the basement membrane, open into deltas consisting of networks of microvillous projections.

Sections show examples of different stages in the formation of the mucous granules in the goblet cells, and there is some evidence that the mucus is produced, stored, and transported inside the closed sacs of the endoplasmic reticulum before transference to the Golgi apparatus where the final product is accumulated.

Aspects of the fine structure of the gall bladder epithelium.

By A. F. Hayward. *University of Glasgow*

An electron microscopic study has been made of the gall bladder epithelium of the mouse. The features observed included the microvillous border, the pronounced intercellular spaces found in methacrylate embedded material, the closely applied subjacent capillaries, and a variety of cytoplasmic granules and vesicles.

Some dense cytoplasmic granules are related to absorption, as they have been shown to increase in size and number after the administration of foreign materials selectively excreted through the bile (e.g. Biloptin, Schering). Groups of large vesicles with pale granular contents, including myeloid figures, found in some epithelial cells are also related tentatively to mucous secretion.

Gall bladders from guinea-pigs were examined after injecting Thorotrast (Testagar) into the lumen *in vivo*. Small vesicles below the apical border were shown to have taken up the colloidal particles, and are therefore considered to result from pinocytosis.

Appositional phagocytosis. By Ian Carr. *University of Glasgow*

Phagocytosis of small particles is accomplished by the ingestion of the particles within membrane-bound vacuoles. In an attempt to elucidate the mechanism of phagocytosis of non-particulate substances, an electron microscopic study has been made of the granuloma produced by the injection of cod-liver oil into the mouse. Three to four weeks after injection a granuloma is present consisting mainly of macrophages with a few giant cells and some fibrous tissue.

The macrophages are closely apposed to the injected fat. The zone of apposition is characterized by disappearance of the cell membrane and the presence of a subjacent area free of organelles. Fat is present within the cell in the form of globules, membrane-bound trabeculae and non-membrane-bound trabeculae. In addition, many fine osmophil granules are present. The appearances suggest that in this experiment phagocytosis of fat occurs by dissolution of the fat in the macrophage membrane, without inclusion in a membrane-bound vesicle.

Changes in the guinea-pig pancreas after cobalt treatment.
By J. D. Lever. *University of Cambridge*

Clear distinction can be made between α- and β-cells in electron micrographs as well as in specifically stained light microscopic preparations of pancreas.

Degranulation and appearances which might be construed as catabolic in significance may be observed in electron micrographs of both exocrine and β-cells but not in α-cells following single cobalt injections. However, the α-cells may be adversely affected on repeated cobalt injection. These findings do not wholly support the suggestion (Volk *et al.*, *Proc. soc. exp. Biol., N.Y.*, 82, 1954) that cobalt specifically and adversely affects the α-cells in the pancreas.

Concentrations of microvesicles and mitochondria have been observed in the terminal or near terminal axoplasm of the arteriolar nerves in the pancreas. In guinea-pigs, after cobalt treatment the majority of these microvesicles contain a filling of an osmiophilic material. The latter finding was discussed in the light of present knowledge on the vasomotor innervation of arterioles.

Cell contacts in the central nervous system.
By A. Peters. *University of Edinburgh*

In recent years, electron microscopy has shown that the plasma membranes of adjacent cells in various tissues are separated by a distance of 150–200 Å. One exception to this is found in the nervous system, where the outsides of plasma membranes come together in such a way that during the formation of myelin a thin, intraperiod line is formed at the site of contact. This observation suggested that the plasma membranes of certain glial cells in the central nervous system might come together in a similar manner to form contacts and an examination of the optic nerves of adult toads (*Xenopus laevis*) and 7- and 14-day post-natal rats has shown this to be the case. In the central nervous system, where the cytoplasm on the outside of the sheath is confined to a small tongue process, an intraperiod line is formed when the outsides of adjacent sheaths come into contact, and when the limiting membrane of a tongue process comes into contact with either a myelin sheath or another tongue process. Similarly, the plasma membranes of certain glial cells and their processes come into contact both with each other and with sheaths and tongues. Such cells and processes are characterized by a well-developed endoplasmic reticulum.

Asymmetry of the node of Ranvier in mammals—an experimental study.
By P. L. Williams and R. Kashef. *Guy's Hospital Medical School, London*

Some previous investigators have observed an asymmetrical form of the nodes of Ranvier in mature peripheral myelinated nerve fibres of mammals. The external and internal diameters and axial length of the proximal bulb, i.e. the bulb nearer the cell body, being greater than those of the distal bulb. The appearance was interpreted in terms of intra-axonal pressures associated with proximo-distal flow of axoplasm, the mechanical properties of the nerve fibre sheaths in the paranodal region and the existence of a 'constriction' at the mid-nodal point.

This hypothesis was tested experimentally by a quantitative study of the asymmetry in (*a*) mature muscle nerves, (*b*) mature cutaneous nerves, (*c*) mature dorsal and ventral nerve roots, (*d*) young growing peripheral nerves, (*e*) regenerates after simple crushing of the mature nerve, (*f*) after crushing neonatal nerves and allowing simultaneous regeneration and continued axial growth, (*g*) after muscle transpositions in young animals, (*h*) in the descending, curved and ascending parts of the recurrent laryngeal nerve. Both fresh-unfixed and fixed-stained preparations (whole amounts of isolated fibres after teasing, and serial sections) of the nerves of rabbits and monkeys were used.

An analysis of the estimations revealed that the polarity of the asymmetry: (i) was unrelated to orthodromic conduction; (ii) bore no constant relationship to the position of the cell body—the theory of flow as a causative factor was therefore rejected; (iii) bore a high correlation to the direction of axial displacement of the Schwann cell during growth—it is therefore suggested that different mechanical conditions operate during myelinogenesis at the 'advancing' and 'trailing' ends of the cell.

Finally a more detailed analysis of the form of the compact myelin sheath and the axon in the paranodal region was made, using serial fresh frozen sections of a muscle nerve, and polarization, phase contrast and interference techniques.

Histochemical detection and localization of oxidative enzymes in the conduction system of the heart. By T. H. Schiebler. *University of Kiel, Germany*

In order to obtain new information on the metabolism of the Purkinje fibres, the occurrence and the distribution of cytochrome oxidase, DPN- and TPN-diaphorase and of some typical substrate specific dehydrogenases were studied in the hearts of calves, pigs and sheep by means of histochemical methods.

All the oxidative enzymes found in the heart muscle also occur in the Purkinje fibres. However, the amount of enzyme demonstrable in Purkinje fibres often differs from that in the muscle fibres. Thus the histochemical reaction for cytochrome oxidase is always, and that for succinic and β-hydroxybutyric acid dehydrogenase usually, less pronounced in the Purkinje fibres than in the heart muscle fibres. DPN-diaphorase, malic, lactate and alcoholic dehydrogenases are usually also found to be more abundant in the Purkinje fibres.

In each species the end product of the histochemical reaction shows a characteristic distribution within the individual Purkinje fibres; in sheep the end products are found to be deposited along the surface of the cell whereas in pigs and calves they are distributed more or less diffusely throughout the cell.

The results obtained might indicate that in Purkinje fibres there is less aerobic metabolism than in the muscle fibres of the heart. Anaerobic glycolysis and its inversion seems to be a most prominent feature of the metabolism of the conduction system of the heart.

The histochemical findings were related to observations on the capillary supply of the heart muscle and of the conducting system.

The specialized conducting tissue of the whale heart: foetal *Balaenoptera physalis*. By T. Summerfield King. *University of Sheffield*

A study has been made of the conducting tissue of foetal whale hearts, using both complete transverse sections of the heart, and sections made from blocks cut from selected portions of the heart.

The sinu-atrial node is identified as an irregular flat plaque in the region of the cranial part of the sulcus terminalis. Both this node and the atrioventricular node are composed of loose networks of pale-staining slender fibres. At the age of these foetuses the ordinary myocardial muscle fibres are themselves slender, and only slightly wider than the nodal fibres.

The atrioventricular bundle is compact, rounded in section, and composed of pale fibres which are very distinct from ordinary cardiac muscle. The limbs of the bundle are very slender and travel by passages through the interventricular muscular septum to reach the endocardium where they become continuous with the Purkinje fibre network.

Further observations on mucopolysaccharides in inflammation. By R. Barer, G. R. Barer and S. Bradbury. *University of Oxford*

In a previous communication (Barer, Barer & Bradbury, *Anat. Soc.* April 1961) we described the distribution of neutral mucopolysaccharides in experimental abscesses in the ear of the rabbit. It was also shown that the polymorphs in the peripheral blood and in pleural and peritoneal exudates showed a considerable increase in PAS activity during the development of an inflammatory process. The skin is not an ideal site for this type of work, since it normally contains a good deal of PAS positive material. Inflammatory lesions and abscesses have therefore been studied in a number of internal organs, including liver, kidney, spleen, omentum, brain and muscle. The types of inflammation have included sterile abscesses produced by turpentine, acute bacterial abscesses (e.g. staphylococcal) and chronic lesions (e.g. tuberculous). Foreign body reactions have also been studied. Observations have been made on both experimental and clinical material. It can be stated in general that an increase in intracellular mucopolysaccharides has so far been found in all cases of inflammation. In acute conditions the polymorphs in the site, and in the inflamed blood vessels show a strong PAS reaction. Localization of the lesion appears to be accompanied by the formation of a 'wall' containing degenerating polymorphs. In chronic inflammations and foreign body reactions increased PAS activity is found chiefly in the cells of the reticulo-endothelial system and in fibroblasts. Certain cells in the spleen give a particularly strong reaction. It appears that the production of neutral mucopolysaccharides is a general and important feature of many types of inflammatory reaction.

The structural pattern of connective tissue as an arrangement for the reception of developing organs. By A. Dabelow. *University of Mainz, Germany*

The well known and widespread diagonal pattern of the collagen fibres of connective tissue has been considered until now in the first place in connexion with its functional importance with regards to stress and strain. To this, however, must be added a second important task. As has been shown by comparative studies, mainly in our Institute, the pattern has an important significance in determining the form and arrangement of later developing organs. The latter activity is demonstrated by: the contorted form of the sweat glands and the proportional behaviour of the straight and the contorted parts; the placing of the glands in the tunica propria of the intestine; localization and early development of the breast (pre- and postnatal); the growth of glands of the tongue and the implantation of the teeth; early development of lobules of fat in the subcutaneous connective tissue. In their mode of evolution all these very different formations show a close correlation with the former structure of the connective tissue, which also determines the first pattern of the blood-vessels. The widespread existence of this pattern makes it probable that widespread casual factors are the basis of their formation. The likely factors were discussed.

Time-lapse phase-contrast cinemicrography of lymphoid tissue in culture. By J. A. Sharp. *University of Leeds*

The appearance and activities of macrophages and lymphocytes in cultures of rabbit lymphoid tissue were shown in a short film, containing the following sequences:

(1) Macrophages and lymphocytes in normal lymphoid tissue.

(2) The behaviour of macrophages and lymphocytes in cultures of lymphoid tissue after antigenic stimulation *in vivo*.

(3) The effect of uraemic rabbit serum (blood urea nitrogens 239·6 and 224·0 mg. %) on the activity of macrophages and lymphocytes in culture.

The delayed rejection of skin homografts in uraemic recipients were discussed with reference to the effect of uraemic serum on lymphoid tissue in culture.

The development of bone-marrow in the human foetus. By J. M. Yoffey and D. Brynmor Thomas. *University of Bristol*

The differentiation of the bone-marrow has been studied in fifty human foetuses immediately after delivery by abdominal hysterotomy. In the earliest stages of its development the bone-marrow consists of dilated, thin-walled sinusoids surrounded by loose mesenchyme—the primary bone-marrow of Hammar (*Anat. Anz.*, 1901, **19**). The humeral marrow presents this appearance during the 10th week of gestation, its cytological composition undergoing a complete transformation during the next 3 weeks, so that by the 14th week the main haemopoietic cell groups are present in roughly equal proportions—erythroid cells, myeloid cells and lymphocytes each accounting for about one quarter of the haemopoietic cell population. There is, however, no organized lymphoid tissue such as occurs characteristically in association with lymphocytopoiesis in other situations. On the contrary the lymphocytes are scattered diffusely among the other marrow cells. We believe that they do not differentiate *in situ* but infiltrate the marrow as suggested by Hammar. We incline to the view that the lymphocytes are carried by the blood from the lymphoid tissues to populate the marrow. This interpretation is not incompatible with our observations on the lymphocyte content of foetal blood (Thomas & Yoffey, *J. Physiol.*, in the Press). In the bone-marrow the lymphocyte may serve as the haemopoietic stem-cell, a role suggested for it by the abundance of cells intermediate in appearance between small lymphocytes and blast cells as well as by the absence of any other numerically adequate stem-cell population. Our observations do not provide support for the view that the mesenchyme of the bone-marrow is the source of haemopoietic stem-cells in this situation.

A study of homologous cancellous bone combined with autologous red marrow after transplantation to a muscular site. By R. G. Burwell. *University of Leeds*

In previous communications it was shown by inserting homografts of tissues into the subcutaneous tissue of the rabbit's ear and studying the response of the draining lymph node, that the principal antigenic component of a homograft of iliac cancellous bone is the red marrow. Marrow-free cancellous bone does not usually elicit an immune response in the regional lymph node (Burwell, R. G., *J. Anat., Lond.*, 1960, **94**).

The finding in rabbits that cancellous bone *per se* is not apparently antigenic has been confirmed in rats using the skin-graft test-system for transplantation immunity. Seventy-four young adult male Wistar rats were challenged subcutaneously with tissues from hooded rats and 3 weeks later were again challenged with a skin homograft from the respective donor. It was found that homografts of marrow-containing cancellous bone evoke transplantation immunity, but homografts of marrow-free cancellous bone do not evoke such immunity.

Homografts of cancellous bone transplanted to a muscular site in the rat form new homograft bone for only a few days and subsequently die; autografts of cancellous bone produce a vigorous and continued new bone formation (Chalmers, J., *J. Bone Jt Surg.*, 1959, **41 B**). In view of the finding that homologous cancellous bone *per se* is not detectably antigenic, the effect of combining homologous iliac cancellous bone (from hooded rats) with autologous femoral red marrow upon the subsequent fate of the graft when transplanted to a muscular site has been studied. Including the control series of experiments, grafts were inserted into the paravertebral muscles of seventy-eight young adult Wistar rats; the animals were killed 2, 6 or 12 weeks after operation.

The natural history of the composite homograft-autograft is similar to (*a*) that of the autograft, and (*b*) that of autologous iliac cancellous bone combined with autologous femoral red marrow.

The findings were discussed from the viewpoints of causative mechanisms and practical applications.

An embryological analysis of certain cardiac abnormalities produced in the offspring of trypan blue injected rats. By G. A. Christie. *University of Glasgow*

The early development of induced cardiac abnormalities has been followed in rat embryos and foetuses. The teratogenic agent used was the diazo dye trypan blue, 1 c.c. of a 1 % aqueous solution of which was injected into the dams at 8½ days of gestation.

An incidence of 11·8 % of cardiovascular defects was observed. Of these, 50 % showed abnormal placing of the bulbo-ventricular part of the cardiac loop. When these had differentiated, the atria were in correspondingly abnormal positions.

This syndrome is very similar to one described by Fox & Goss (*Anat. Rec.*, 1955–56–57, **121**, **124**, **129**) which comprised: lateral shift of the atria; alterations of heart position and shape; rotation of the ventricles; interventricular septal defect; transposition of the major arterial trunks.

The evidence provided by this study would tend to substantiate their hypothesis that these abnormalities are due to early abnormal looping of the cardiac tube, with consequent rotation and displacement of the atrial chamber prior to its differentiation.

The nitrite sensitivity of rat haemoglobin: the effect of teratogenic and related dyes. By W. K. Metcalfe. *University of Bristol*

The sensitivity of the haemoglobin of rats to nitrites can readily be increased by starvation, tumour implantation, pregnancy and gamma radiation. In all except the last of these the haemoglobin stability can be restored to normal by the administration of riboflavine. Animals deficient in riboflavine are known to lose weight and, if pregnant, either to resorb their foetuses or to produce an increased proportion of foetal abnormalities. At the last meeting of the Society, similar changes were reported by Beck, following the administration of a commercial sample of trypan blue. It seemed possible that this preparation might produce its effects by interference with the flavoprotein constituents of the respiratory chain. It was felt that experiments along these lines might perhaps help to elucidate the basic mechanism of the nitrite sensitivity reaction.

It was found that trypan blue (Williams), in sufficient dose, did cause, within 48 hr., an increase in haemoglobin sensitivity. The change was reversed by quite small doses of riboflavine (2 mg.) provided that 7 days had elapsed after the last dye injection. Five days after the last injection, even a large dose of riboflavine phosphate (25 mg.) took 24 hr. to restore the stability of the haemoglobin. Further shortening of the interval made even this dose of riboflavine ineffective.

In an attempt to define the active chemical group, compounds structurally similar to trypan blue have also been tested.

Observations on trypan blue toxicity By F. Beck.
Department of Anatomy, University College, Cardiff

Groups of adult Wistar rats were injected subcutaneously with trypan blue (T.B.) preparations at dosage levels adjusted so as to produce either death within 24 hr. following a single massive dose or chronic intoxication following repeated smaller doses. Histological sections of liver, kidney, spleen and suprarenal were examined for each group. The findings were discussed in relation to the possible causes of death.

According to Metcalf (personal communication) subcutaneous injection of T.B. preparations increase the sensitivity of red cells to methaemoglobinization, but riboflavine and flavine mononucleotide protect to some extent against this injurious effect. Accordingly, T.B. preparations were administered simultaneously with riboflavin or flavine mononucleotide and this combination tested for toxic and teratogenic properties. The results were presented.

Since H-acid is an important constituent of the T.B. molecule, it was also tested on pregnant rats and found to be completely non-teratogenic.

The transport of trypan blue by the seminiferous tubules of the rat testis. By E. W. Macmillan and E. J. Clegg. *University of Liverpool*

At operation in a random series of mature male rats 0·5 % trypan blue solution was introduced, by retrograde injection via the rete testis into the adjoining seminiferous tubules. The entry of the dye into the lumina of the tubular system, though visible to the naked eye, was confirmed in each case by low-power binocular microscopy.

The animals were sacrificed at intervals from 22 hr. to 32 days, the testis and epididymis inspected and then examined histologically.

The dye is rapidly evacuated by the testis into its excurrent duct system. Twenty-two hours after operation the ductuli efferentes and the caput epididymidis are deeply stained, and remain so, clearly defined from the testis and the rest of the epididymis long after the remnants of the dye have been transported into the proximal ductus deferens.

Histological examination confirms that the lining cells of the efferent ductules and the caput epididymidis show aggregations of granules of the dye. On the other hand, the Sertoli cells, the germinal cells, and the lining epithelium of the rete testis contain no granules. The contrast at the junction of the efferent ductules and the rete testis is clear cut.

It would appear that the integrity of the germinal epithelium and the speed of the evacuation of the dye allows little opportunity for the Sertoli cells to exhibit vital staining. On the other hand, the special property of the cells lining the proximal duct system of the testis is demonstrated. There is little doubt that the dye enters the lining cells from the sperm stream directly.

Observations on the uptake of trypan blue by the seminiferous tubules of the rat testis. By E. J. Clegg and E. W. Macmillan. *University of Liverpool*

By the technique described above a 0·5 % solution of trypan blue was introduced into the seminiferous tubules of both testes of adult rats, this procedure being immediately followed by unilateral ligation of the ductuli efferentes.

Animals were killed at intervals from 5 to 32 days after operation. While aggregations of dye were not found in the Sertoli cells of testes in which the ductuli efferentes remained intact, they were present in all except one instance in animals in which ligation had been performed. The definition and intensity of colour of the granules was at a maximum 32 days after injection.

Further experiments in which the dye was introduced into the tubules of testes, previously subjected to various operative procedures (cryptorchidism, interruption of ductuli efferentes) were described.

The vascular architecture of mammalian bone cortex. By M. Brookes. *University of Liverpool*

The vascular patterns within tubular bone cortex of the posterior limb skeleton of mammals belonging to several mammalian orders have been investigated, principally by vascular injection methods utilizing Thorotrast or Micropaque followed by microradiography, or indian ink suspension followed by examination of Spalteholz preparations.

The results show that in post-natal bones, three distinct vascular fields can be identified in bone cortex, but in foetal material only one is represented. The arrangement of blood vessels within the three vascular components does not appear to be directly related to the mechanical stresses sustained by a bone during life. It is suggested that the patterns of the three vascular fields, primary, secondary and tertiary, can be correlated with the mode of formation of periosteal, endochondral and endosteal bone respectively.

Repair of a bone gap. By P. McClements, R. W. Templeton and J. J. Pritchard. *Queen's University, Belfast*

The influence of the presence or absence of periosteal continuity on the mode and speed of repair of a bone gap has been studied in rats.

In the presence of an intact bridge of periosteum, gaps of any width in a rib were repaired inside 3 weeks. The periosteum in the gap became oedematous and infiltrated with polymorphs within a few hours, and then the cambial cells proliferated very rapidly, giving rise to a thick layer in the midst of which chondroblasts and osteoblasts differentiated. A rod of cartilage interspersed with areas of cancellous bone was soon formed, and then the cartilage was replaced and the bone remodelled to produce a new rib segment. Localized cautery damage to the periosteum inhibited bone and cartilage formation indefinitely in the immediate vicinity of the injury.

In contrast with this, in the absence of a periosteal bridge, a bony gap initially only 2 mm. wide in the third metatarsal was incompletely bridged with new bone in 20 weeks. The mechanism of repair was quite different. Muscle contraction soon reduced the gap to 0·5 mm. The gap was then invaded by fibroblasts which formed tracts of dense fibrous tissue, usually enclosing a slit-like cavity. The fibrous tissue later appeared to be under compression, and it was transformed into fibro-cartilage. Ossification then slowly advanced into the fibro-cartilage from the open medullary cavities on each side.

It was concluded that the presence or absence of a periosteal bridge is a decisive factor in determining the speed and mode of fracture repair.

The growth of the tail in the rat. By D. A. J. Keegan and T. J. Harrison. *Queen's University, Belfast*

Tail growth was studied post-natally in Wistar rats and in tails transplanted subcutaneously into the abdominal wall of the mother.

Radiographic, histologic, and autoradiographic techniques were used. Growth of the whole tail relative to the sacral and pre-sacral parts of the vertebral column, of different regions of the tail, and of individual tail vertebrae, were studied.

Tail growth was found to be most rapid during the first 80 days of life and decelerated markedly after 100 days of age, but some growth was still taking place at the end of 200 days. Between the 7th and 200th day of life the tail increased six times in length, while the sacrum and vertebral column each increased four times in length. The middle region of the tail grew faster than either the proximal or distal regions. In individual tail vertebrae more growth occurred at the caudal end. In tail transplants the anulus fibrosus became ossified, producing structures very similar to the disc bones of the sacrum.

The vascularity of the developing pulp in teeth of limited growth. By D. Adams. *University of Edinburgh*

The capillary systems in the pulps of kittens' teeth were studied at various stages of development using perfusion methods. The most interesting feature of these pulps was the presence of a capillary network lying between the odontoblasts and the predentine, once dentine production has started, an arrangement similar to that found in the persistently growing teeth of rodents. This close relationship of capillaries to predentine was not found in the root region of the kitten's teeth, where the odontoblasts did not become pseudostratified as in the crown. When the teeth had erupted, the close relationship between blood vessels and predentine is lost. Two factors appear to determine the invasion of the odontoblast layer by blood vessels, namely, the rapid production of dentine and pseudostratification of the odontoblasts.

Electron microscope study of demineralized dentine. By H. W. Noble and A. F. Carmichael. *University of Glasgow Dental School*

Ultra-thin sections of demineralized human dentine were prepared for examination under the electron microscope. Satisfactory results were obtained only when the tooth was correctly sectioned immediately after extraction in order to permit adequate penetration of the processing solutions. The electron micrographs revealed the characteristic orientation of the collagen fibres of the intertubular dentine matrix. The acid mucopolysaccharide ground substance in which these fibres are believed to be embedded and which forms the collagen-free matrix of the peritubular translucent zone was lost during the process of demineralization.

A dense granular appearance was frequently observed in the predentine zone of developing matrix. The granules, which were frequently fused into large aggregates, varied from 400 to 1000 Å in diameter. The protoplasmic core of the dentinal process of the odontoblast cell was seen to be very sensitive to variations in processing technique. When well preserved it was observed to contain many longitudinally arranged fibrils. The membrane surrounding this process was less readily destroyed and appeared to be thicker than a normal cell membrane.

The brain stem in a case of dicephaly in a newborn human foetus. By Francis J. Warner, M.D. *Philadelphia, U.S.A.*

In this dicephalous newborn human foetus were noted two separate cerebra, two diencephala, and two separate midbrains, as well as a conjoined rhombencephalon. The caudal extremities of the mesencephala were fused at the level of the rostral extremity of the pons. The midsagittal plane of the conjoined rhombencephalon represented the line of fusion of the brain stems of the two cerebra. In the respective brain stems of each head member a median raphé was noted, which represented the median sagittal plane of these respective brain stems.

The outer walls of the conjoined rhombencephalon were relatively normal in appearance and contained the fibre tracts, nuclei and nerve roots of the normal human rhombencephalon. Lying between the two median raphés a median area of the conjoined rhombencephalon was noted.

Other abnormalities included a duplication of the medial longitudinal bundle, the medial lemniscus and the pyramidal tract, as well as the pyramids of the medulla, on each side of the median raphés of the respective brain stems of each member head. There was no evidence of a decussation of the pyramidal tracts in the medulla.

'Milk spots' in the meninges of newborn and stillborn infants. By J. Watt. *University of Liverpool*

In a series of post-mortems on stillborn and newlyborn infants, 'milk spots' were found in the meninges of the brain in about 50 % of cases. They occurred in nearly two-thirds of all stillbirths and in about one-third of all neonatal deaths.

The histological features varied from typical arachnoidal cell clusters to a very much more fibrous lesion in which capillary vessels sometimes occurred.

Arachnoidal cell clusters have been described in man and in various animals and are generally accepted as a manifestation of advancing age. Their absence in very young animals has been cited in support of such a conclusion (Weed, *Johns Hopkins Hosp. Bull.*, 1920, **31**). The significance of 'milk spots' in the human foetus was discussed in relation to this theory.

Some observations on the arteries of the bovine umbilical cord. By P. Bacsich, H. Boyd and A. Young. *University of Glasgow*

The present investigation forms part of two larger inquiries now in progress, one being concerned with the incidence and associated physiological and pathological factors of embryonic death in cattle, the other with the comparative anatomy of the vessels of the umbilical cord, with special reference to the presence or absence of a transverse communicating branch between the umbilical arteries, which in a juxta-placental situation form a constant feature of the human cord.

Examination of 100 bovine umbilical cords revealed the existence of a transverse branch in the lower third of the cord, big enough to provide a functionally useful collateral pathway, in 60 cases. In a further 20 cases this vessel, though present, was either of uneven calibre or too small to have any definite circulatory significance; while in the remaining 20 cases there was no communication at all between the arteries within the cord.

Bacsich & Smout (*J. Anat., Lond.*, 1938, 72) postulated that such a transverse communicating branch between the umbilical arteries is the result of necessity, has a pressure regulating function and subserves the specialized needs of the human placenta. More recently this view was not only endorsed, but further amplified by later workers. On the other hand, in the earlier literature the occasional presence of a transverse communicating branch between the arteries of the bovine cord received but casual mention, and seemed to have been considered of no significance.

The present findings indicate that the transverse branch is not an exclusive feature of the human cord, but may appear, probably as a result of parallel evolution, in other species also.

Sex chromatin analyses in a mentally defective population. By W. R. Breakey. *Queen's University, Belfast*

Two hundred and ninety-seven mentally defective patients (157 males and 140 females) were examined by the buccal smear technique for the presence of sex chromatin bodies (of Barr) in the nuclei. Counts in male subjects had a modal value of 0, a proportion giving higher values, with a mean of 4·44; counts in female subjects had a mean of 31·25 and a standard deviation of 11·48 with a normal distribution curve. These values are within the accepted ranges of normal. One phenotype male patient with partial colour blindness, had 37% single and 7% double chromatin bodies in 700 buccal cells. Blood smears showed single drumsticks in 12 out of 795 cells, one of which showed double drumsticks. A chromosome analysis from cultured white blood cells showed the chromosomal count to be 48, with $XXXY$ sex chromosomes. The distribution of counts was 46, 46, 47, 48, 49 and >49 and the frequency 49 in 2, 2, 13, 55, 6 and 1 cells respectively. Testicular biopsy showed a few degenerate tubules and a great increase of interstitial cells in both testes. The diagnosis of Klinefelter's syndrome was confirmed. Chromatin analysis from buccal smears of his parents and 6 sibs were normal. Only his mother, of those relatives tested, showed slight defects with the Ishihara charts.

It is suggested, by reason of the colour-blindness findings, that in oogenesis, due to double non-disjunction, three X-chromosomes were contributed by the mother and that two or three of these carried recessive colour-blindness mutants. It is further suggested that it was this dosage-effect which gave rise to the increased degree of colour-blindness in the son over that in the mother, whose chromosomes carried one or two colour-blindness mutants.

Muscular variation in the primate shoulder. By E. H. Ashton and C. E. Oxnard. *University of Birmingham*

A study of 28 genera of Primates (including man), has shown that several quantitative features of the shoulder muscles vary according to the method of locomotion. Thus, for instance, of the muscles which in subhuman Primates contribute to the propulsive move-

ment, m. latissimus dorsi is more powerfully developed and more cranially directed in brachiators (i.e. apes) than in quadrupedal monkeys (e.g. *Papio*, *Saimiri*). Again, in brachiators, the pectoral muscles are directed more laterally than in quadrupeds. In addition, the muscles responsible for raising the forelimb (m. deltoideus; m. trapezius and the caudal part of m. serratus magnus) are relatively bigger in brachiators than in quadrupeds. In certain monkeys ('semibrachiators'—e.g. *Lagothrix*, *Colobus*) which move both quadrupedally and by swinging from the forelimbs, the muscular features are intermediate between those characteristic of quadrupeds and brachiators. In man, the shoulder muscles show a unique balance of these anatomical features.

The contrast in muscular features between those Prosimii which move quadrupedally (e.g. *Lemur*) and those which frequently hang from the extremities (e.g. *Perodicticus*), parallels that between quadrupeds and brachiators in the Anthropoidea.

Observations on the muscles and tendons of the medial aspect of the sole of the foot. By B. F. Martin. *University of Sheffield*

From a study of 32 foot dissections some observations were made, relative to the medial aspect of the sole of the foot, which elaborate existing anatomical data, and other observations revealed inaccuracies in current accounts.

The main findings were as follows. The medial intermuscular septum generally consists of three bands which subdivide prior to attachment to the tarsus. The middle band gives origin to the superficial fibres of flexor hallucis brevis, whilst deep to the muscle, the metatarsal extension of tibialis posterior passes between the two divisions of the band and there gives off a triangular fold which bends medially to gain the deep surface of the muscle. The tendon of flexor hallucis longus first lies lateral to the medial intermuscular septum and then runs obliquely alongside the lateral head of flexor hallucis brevis (and not between the heads of that muscle, as normally described), before gaining the inter-sesamoid interval; it is, in fact, clamped to the lateral head by the distal band of the medial intermuscular septum. The insertion of tibialis posterior was studied, and a bursa, about 2 in. long, was consistently found on the deep aspect of its metatarsal extension.

The importance of form in the bladder neck region. By S. A. Vincent. *Queen's University, Belfast*

When the bladder neck is raised in relation to the rest of the bladder the outlet closes. This appears to depend on the form and consistency of the tissue mass surrounding and below the outlet.

This investigation was concerned with a study of the changes of form of this tissue mass in response to neighbouring mechanical influences, as distinct from its ability to change shape by means of its own intrinsic musculature.

Three hundred and fifty fresh unfixed human bladders were examined in a machine which produced changes in the form of the bladder neck.

In living patients, all departures from normal in this form were studied in relation to the symptoms with which they were associated.

Extrinsic factors capable of *producing* abnormal forms were investigated and methods of restoring the normal form by changing these factors were described. These methods included the use of an appliance which elevated the pelvic floor and thus closed the male or female bladder outlet. In patients with certain types of incontinence their own pelvic floor muscles produced the required elevation after a short course of treatment with the appliance.

The significance of these findings was discussed.

Unilateral renal agenesis with associated unicornuate uterus in a cat.
By F. C. Robson and R. J. Scothorne. *King's College, Newcastle upon Tyne*

A number of genito-urinary anomalies was discovered incidentally in an experimental cat.

The right kidney and ureter were totally absent. No sign was found in serial microscopic sections of a right ureteric orifice in the bladder. Because of the contraction of the bladder no definite statement can be made about the trigone. Left kidney and ureter were essentially normal.

Of right Mullerian duct derivatives, only the rostral end of the Fallopian tube was present, lying rostrally to the right ovary. A narrow tube, closed at each end and lined by simple columnar epithelium, was seen in serial microscopic sections lying beside the right Fallopian tube, and was interpreted as representing the persisting rostral end of the right Wolffian duct (epoophoron). No sign of Gärtner's duct was found, in an interrupted series of sections through the vagina.

The right ovary was present and appeared normal, both grossly and microscopically, being in fact larger than that on the left side.

A ligamentous band extended caudally from the caudal pole of the ovary to the deep inguinal ring and was interpreted as representing the gubernaculum. It had no attachment to the unicornuate uterus.

The vagina and external genitalia appeared normal.

The suprarenal glands were normal in position and microscopic structure. In explanation of the embryological origin of these defects it is suggested: (i) that the caudal part of the right Wolffian duct failed to develop (or to persist), with consequent agenesis of the right ureteric bud and of the metanephros; (ii) that failure of development of all but the rostral end of the right Mullerian duct was secondary to failure of Wolffian duct development.

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